Long-term outcome and prognostic value of Ki67 after perioperative endocrine therapy in postmenopausal women with hormone-sensitive early breast cancer (POETIC): an open-label, multicentre, parallel-group, randomised, phase 3 trial

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Summary

Background Preoperative and perioperative aromatase inhibitor (POAI) therapy has the potential to improve outcomes in women with operable oestrogen receptor-positive primary breast cancer. It has also been suggested that tumour Ki67 values after 2 weeks ($Ki67_{2W}$) of POAI predicts individual patient outcome better than baseline Ki67 ($Ki67_{B}$). The POETIC trial aimed to test these two hypotheses.

Methods POETIC was an open-label, multicentre, parallel-group, randomised, phase 3 trial (done in 130 UK hospitals) in which postmenopausal women aged at least 50 years with WHO performance status 0–1 and hormone receptor-positive, operable breast cancer were randomly assigned (2:1) to POAI (letrozole 2·5 mg per day orally or anastrozole 1 mg per day orally) for 14 days before and following surgery or no POAI (control). Adjuvant treatment was given as per UK standard local practice. Randomisation was done centrally by computer-generated permuted block method (variable block size of six or nine) and was stratified by hospital. Treatment allocation was not masked. The primary endpoint was time to recurrence. A key second objective explored association between Ki67 (dichotomised at 10%) and disease outcomes. The primary analysis for clinical endpoints was by modified intention to treat (excluding patients who withdrew consent). For Ki67 biomarker association and endpoint analysis, the evaluable population included all randomly assigned patients who had paired Ki67 values available. This study is registered with ClinicalTrials.gov, NCT02338310; the European Clinical Trials database, EudraCT2007-003877-21; and the ISRCTN registry, ISRCTN63882543. Recruitment is complete and long-term follow-up is ongoing.

Findings Between Oct 13, 2008, and April 16, 2014, 4480 women were recruited and randomly assigned to POAI (n=2976) or control (n=1504). On Feb 6, 2018, median follow-up was 62·9 months (IQR 58·1–74·1). 434 (10%) of 4480 women had a breast cancer recurrence (280 [9%] POAI; 154 [10%] control), hazard ratio 0·92 (95% CI 0·75–1·12); p=0·40 with the proportion free from breast cancer recurrence at 5 years of 91·0% (95% CI 89·9–92·0) for patients in the POAI group and 90·4% (88·7–91·9) in the control group. Within the POAI-treated HER2-negative subpopulation, 5-year recurrence risk in women with low $Ki67_{B}$ and $Ki67_{2W}$ (low–low) was 4·3% (95% CI 2·9–6·3), 8·4% (6·8–10·5) with high $Ki67_{B}$ and low $Ki67_{2W}$ (high–low) and 21·5% (17·1–27·0) with high $Ki67_{B}$ and $Ki67_{2W}$ (high–high). Within the POAI-treated HER2-positive subpopulation, 5-year recurrence risk in the low–low group was 10·1% (95% CI 3·2–31·3), 7·7% (3·4–17·5) in the high–low group, and 15·7% (10·1–24·4) in the high–high group. The most commonly reported grade 3 adverse events were hot flushes (20 [1%] of 2801 patients in the POAI group *vs* six [<1%] of 1400 in the control group) and musculoskeletal pain (29 [1%] *vs* 13 [1%]). No treatment-related deaths were reported.

Interpretation POAI has not been shown to improve treatment outcome, but can be used without detriment to help select appropriate adjuvant therapy based on tumour Ki67. Most patients with low $Ki67_{B}$ or low POAI-induced $Ki67_{2W}$ do well with adjuvant standard endocrine therapy (giving consideration to clinical–pathological factors), whereas those whose POAI-induced $Ki67_{2W}$ remains high might benefit from further adjuvant treatment or trials of new therapies.

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Research in context

Evidence before this study

Longstanding experimental evidence from 1989 led to the hypothesis that short duration, presurgical endocrine therapy for early oestrogen receptor-positive breast cancer might improve clinical outcome. We carried out a PubMed search for relevant clinical studies published from Jan 1, 1989 until Dec 31, 2019 using the terms "neoadjuvant endocrine", "breast cancer", "clinical trial", and "presurgical and endocrine therapy". No reasonably sized randomised trial addressed this issue by the time POETIC commenced recruitment in 2008. Subsequently, a randomised clinical trial reported that depot progesterone for 5–14 days before surgery improved outcome in node-positive early breast cancer. Before the initiation of POETIC, two small clinical neoadjuvant trials, IMPACT and Z1031, reported that tumour Ki67 2–4 weeks after starting preoperative endocrine treatment predicted outcome better than baseline Ki67. POETIC was designed to establish whether the gain in prognostic accuracy merited routine application of presurgical endocrine therapy for this purpose. An additional PubMed search was done with "Ki67" added to the above search terms. One small study of low dose tamoxifen was identified, but this did not substantially add to the earlier evidence. Another modestly sized trial used to triage patients with 2–4 week Ki67>10% to chemotherapy and reporting the long-term outcome for those less than 10%, has led to a larger ongoing trial. One other large ongoing trial applies 10% as a cutoff at 2 weeks of tamoxifen or an aromatase inhibitor for directing patients to different adjuvant therapy. The concept of complete cell cycle arrest has been developed as an additional possible cutoff for on-treatment Ki67.

Added value of this study

Results from POETIC suggest that 2 weeks' preoperative endocrine therapy makes no perceptible improvement in long-term outcome, but was nevertheless a safe treatment practice. The trial confirmed the low risk of recurrence for those with a low baseline Ki67. In patients with a high baseline Ki67 value (>10%) a biopsy 2 weeks after starting preoperative endocrine therapy provides additional clinical utility by predicting long-term outcomes. The trial documents the relationship of 2-week Ki67 with risk of recurrence for estimating whether the prognosis of individual patients is sufficiently good on endocrine therapy alone or whether additional treatment such as chemotherapy or new targeted therapies should be considered.

Implications of all the available evidence

The data show no reason for short-term presurgical treatment to be applied for its direct therapeutic potential, but support prescribing an aromatase inhibitor for the short-term period before breast cancer surgery in oestrogen receptor-positive tumours with a high proliferation rate to derive information on early endocrine responsiveness that can be used to predict a patient's 5-year prognosis on standard adjuvant therapy. The clinical manoeuvres to incorporate this in the patient pathway with reliable quality assured Ki67 are straightforward and the measurement of Ki67 is inexpensive, potentially making this an attractive approach to estimating the prognosis of patients with early breast cancer.

Introduction

The POETIC (Peri-Operative Endocrine Therapy—Individualising Care) trial was designed to address two important hypotheses in the treatment of post-menopausal women with oestrogen receptor-positive early breast cancer.

The first was that short duration presurgical endocrine therapy might improve clinical outcome. This hypothesis was plausible because 2 weeks' preoperative endocrine therapy had been shown to markedly reduce proliferation in human breast cancer as measured by Ki67.[1,2] Longstanding experimental evidence had shown that the stimulatory effect of surgery on the growth of metastases in mice could be inhibited by perioperative endocrine therapy.[3,4] Any improvement in long-term outcome following short exposure to preoperative or perioperative endocrine therapy would be achieved with no additional toxicity or resource implications and be of considerable clinical importance.

The second hypothesis concerned identifying which patients with hormone receptor-positive early breast cancer have a sufficiently good prognosis such that standard of care medical treatment, often comprising adjuvant endocrine therapy alone, was sufficient and which group should be considered for additional therapies. Traditional approaches to this problem had used standard prognostic parameters including size, grade, nodal involvement, and age, often integrated into a prognostic tool (eg, Nottingham Prognostic Index,[5] Adjuvant Online,[6] NHS PREDICT[7]), but these merely provided the predicted probability of benefit for a patient population with given tumour and demo-graphic characteristics. More recently, genomic platforms have been developed aimed at providing more accurate prognostic and predictive information for the individual patient.[8,9] However, these genomic tests are expensive, by no means universally available, and differ among themselves in terms of the information they provide.[10]

A simple test which predicts outcome after short duration preoperative endocrine therapy could therefore be helpful in accurately selecting appropriate treatment in the individual patient, if it incorporated an in-vivo response to aromatase inhibitor. A small neoadjuvant trial (IMPACT) had already suggested this might be feasible: results showed that tumour Ki67 after 2 weeks ($Ki67_{2w}$) of endocrine treatment predicted outcome better than at baseline ($Ki67_{B}$), remaining significant in multivariable analysis, whereas $Ki67_{B}$ did not.[11,12] Similar

results have subsequently been reported from another small trial comparing letrozole with tamoxifen[13] and from a further trial comparing anastrozole, letrozole, and exemestane with one another.[14] POETIC, with a much larger patient population, aimed to build on these findings to provide the definitive clinical evidence to inform future practice.

Methods

Study design and participants

This open-label, multicentre, parallel group, randomised, phase 3 trial recruited participants from 130 UK hospitals (appendix p 25–27). Eligible patients were postmenopausal women (aged at least 50 years with amenorrhoea for more than 12 months, bilateral oophorectomy or hysterectomy, or had been on hormone replacement therapy within the previous 12 months, and with follicle-stimulating hormone concentrations in the postmenopausal range if aged less than 55 years) with oestrogen receptor-positive or progesterone receptor- positive (Allred ≥3, H-score ≥2, or ≥1% of positive cells, assessed in local pathology laboratories), HER2-positive or HER2-negative (assessed locally), operable primary breast cancer and no evidence of metastatic spread investigated according to local guidelines. If palpable, a tumour of any size was sufficient, otherwise requiring an ultrasound size of at least 1·5 cm. Women required WHO performance status 0–1 and an indication for standard adjuvant endocrine therapy. Required staging investigations were according to local practice with no additional trial specific investigations. Exclusion criteria were typical for this patient population. Previous endocrine therapy or chemotherapy was not allowed, nor was concurrent use of hormone replacement therapy or any other oestrogen-containing medication (within 4 weeks of randomisation). No previous use of oestrogen implants at any time, current, continuous, long-term systemic steroid usage, or treatment with an unlicensed or investigational drug within 4 weeks of randomisation was allowed. Patients with invasive malignancy diagnosed within the previous 5 years or any severe co-incident medical disease were ineligible (appendix p 1).

Patients provided written informed consent before enrolment. POETIC was sponsored by the Institute of Cancer Research (ICR) and Royal Marsden NHS Foundation Trust and approved by the London–South East Research Ethics Committee (reference 08/H1102/37) and managed and analysed by the ICR Clinical Trials and Statistics Unit (ICR-CTSU; appendix p 1 for study oversight details). The protocol is in the appendix.

Randomisation and masking

Participants were randomly allocated (2:1) to perioperative aromatase inhibitor (POAI) treatment or no perioperative treatment (control) by computer-generated permuted block method (variable block size six or nine) derived centrally by ICR-CTSU using its dedicated randomisation system, stratified by hospital. To randomly assign a patient, staff at the recruiting site telephoned ICR-CTSU and thus had no knowledge of future treatment assignment. The allocation ratio weighted trial information to study of biological perioperative drug effects, in particular to assess how these effects relate to long-term outcome. No placebo was used; clinicians and patients were not masked to treatment allocation, but central laboratory staff were masked.

Procedures

POAI was a non-steroidal aromatase inhibitor in standard dosage (oral anastrozole 1 mg per day or oral letrozole 2·5 mg per day); choice of agent was declared by each participating hospital at trial outset. Before randomisation, all patients had excisional surgery prebooked for around 2 weeks (minimum 10 days) later to ensure timing of surgery was not biased by treatment allocation. POAI was to commence immediately after randomisation allowing duration of treatment before surgery to be as close as possible to 14 days. If surgery was delayed, the pretreatment duration was extended. Treatment continued without interruption until 14 days after surgery.

See Online for appendix

All non-trial adjuvant therapy, laboratory investigations, and disease staging were established on clinical grounds according to standard of care local practice (appendix p 1). All patients had pretreatment mammography and breast ultrasound according to local diagnostic practice. In December, 2010, the independent data monitoring committee expressed caution relating to the potential influence of POAI therapy on tumour grade measured at surgery. In February, 2011, a letter to investigators, followed by an approved protocol amendment, recommended that local multidisciplinary teams gave due consideration to other factors, including pretreatment grade on diagnostic core where available, when considering use of adjuvant chemotherapy.

Follow-up data were submitted annually to ICR-CTSU; disease-related events, second cancers and deaths were reported on occurrence. There was no specific safety endpoint. Adverse event data were restricted to three menopausal symptoms (hot flushes, sweating, and musculoskeletal pain) at baseline, surgery, and at follow-up 2 weeks postsurgery (assessed using National Cancer Institute Common Terminology Criteria for Adverse Events version 3) as the safety profiles of the aromatase inhibitors used were well established. Serious adverse events were reported or recorded (as per protocol). Participants were able to withdraw from the trial at any time for any reason.

Formalin-fixed paraffin-embedded tissue samples were required before randomisation (baseline) and at surgery. Baseline samples could be a core-cut diagnostic biopsy, a subsequent research core-cut biopsy, or sections from the diagnostic sample. At surgery, samples could be either core biopsies or sections cut from the routine excision.

Tissue samples were processed, stored, and analysed for Ki67 staining centrally in the Ralph Lauren Centre for Breast Cancer Research at the Royal Marsden NHS Foundation Trust. Ki67 was analysed immunohistochemically in a core biopsy taken at baseline ($Ki67_B$), and in either a core biopsy or the excision biopsy taken at surgery ($Ki67_{2W}$), and was estimated as the percentage of cancer cells staining positive. We used MIB1 as the primary antibody to Ki67 and detection was done with the REAL EnVision system, both from DAKO (Glostrup, Denmark until 2016; now Agilent Technologies, Didcot, UK). Scoring was according to methodology including between-batch quality control procedures as described by the International Ki67 in Breast Cancer Working Group Party.[15] Analysis of 2-week samples from the control group was restricted to a randomly selected subset since minimal change from baseline was expected.[16]

Outcomes

The primary endpoint was time to recurrence (time from randomisation to local, regional, or distant tumour recurrence or death from breast cancer without previous notification of relapse) with second primary cancers and intercurrent deaths censored. Secondary clinical endpoints included relapse-free survival (as per time to recurrence but also including deaths from any cause as events), time to local recurrence (time from randomisation to first confirmed local recurrence, censoring at previous distant recurrence, second primary cancer, or death), time to distant recurrence (time from randomisation to first confirmed distant recurrence or breast cancer death without previous relapse, censoring at second primary cancer or intercurrent death) and overall survival (time from randomisation to death from any cause). Breast cancer-free survival duplicated the definition of time to recurrence, and was listed in the protocol in error.

Ki67 was evaluated as a biomarker in relation to its effect on predicting disease outcomes (one of the trial's two key objectives) and as the molecular secondary endpoint to assess proliferation rate at baseline ($Ki67_B$) and at surgery ($Ki67_{2W}$), thus assessing the impact of POAI. The additional molecular secondary endpoint of gene expression profile at core biopsy and at surgical excision is not reported here as data analysis is ongoing.

Statistical analysis

The sample size assumed the proportion of patients with recurrence by 5 years would be low (approximately 10%) given known recurrence rates for similar populations.[17,18] With 4350 patients it would be possible to detect a 3% improvement in time to recurrence at 5 years (10% to 7% recurrences) with 91% power (two-sided α of 5%). The sample size was increased originally from 4000 to 4350 patients to allow for underestimation of the relapse rate potentially owing to patients dying from other causes before breast cancer relapse. This change was endorsed by the trial steering committee and independent data monitoring committee and managed via a protocol amendment approved on Dec 31, 2012.

Analyses relating to clinical endpoints were done according to modified intention-to-treat—removing patients who subsequently withdrew consent for use of data. For analyses that assessed the predictive value of $Ki67_B$ and $Ki67_{2W}$, the population was defined as all randomly assigned patients who had paired Ki67 values available. Patients who did not have primary breast surgery as planned were censored at the date of that decision.

Baseline demographic details, tumour characteristics, adjuvant treatment, and Ki67 data are presented with descriptive statistics. Protocol compliance between treatment groups (time from randomisation to surgery and number of inpatient days for surgery) was compared using Wilcoxon rank-sum tests; differences in tumour grade at surgery were assessed using a χ^2 test for trend in prespecified analyses. Worst grade of adverse events and serious adverse reactions to POAI were summarised descriptively. $Ki67_B$ and $Ki67_{2W}$ were reported by HER2 status. Analysis of percentage change between $Ki67_B$ and $Ki67_{2W}$ used Wilcoxon sign rank tests within treatment groups and Wilcoxon rank-sum test between treatment groups. In a post-hoc exploratory analysis, following initial planned analyses on the trial data, a multivariable logistic regression model was created, using a forward stepwise approach, to determine factors affecting chemotherapy use.

For survival-related endpoints, Kaplan-Meier curves were plotted and treatment groups compared with the log-rank test. Hazard ratios (HRs) and 95% CIs were calculated within Cox proportional hazards regression models, with HRs of less than one taken to favour POAI. The proportional hazards assumption was assessed using Schoenfeld residuals and was found to hold. Comparisons between treatment groups were made with and without adjustment for progesterone receptor status (positive, negative, unknown), HER2 status (positive, negative, unknown), presurgical tumour grade (G1, G2, and G3), pathological tumour size (continuous), presurgical histological type (ductal, lobular, special type), nodal status (N0, N1–3, and N4+), age at randomisation (continuous) and vascular invasion (yes, no). Subgroup analyses were done for baseline clinical characteristics and presented using a forest plot.

Associations between $Ki67_B$ and $Ki67_{2W}$ and time to recurrence were done separately in the POAI and control groups with the principal focus being to study the on-treatment effect of POAI. A post-hoc analysis of all patients combined for $Ki67_B$ was included for completeness. Assessment of Ki67 in the control group was considered of low additional value because patients were not exposed to perioperative treatment and because of the lack of association between POAI and time to recurrence. Survival analysis included adjustment for clinical factors as mentioned previously, except for HER2 status which

was a stratifying factor. HER2-positive tumours have a different pattern of recurrence and were typically additionally treated with specific HER2-targeted therapy. To explore associations between Ki67 and disease outcome in the POAI group, Ki67 scores were dichotomised and patients divided into four groups as follows: low–low ($Ki67_B$ and $Ki67_{2W}$ <10%); high–low ($Ki67_B$ ≥10%, $Ki67_{2W}$ <10%); high–high ($Ki67_B$ and $Ki67_{2W}$ ≥10%); and low–high ($Ki67_B$ <10%, $Ki67_{2W}$ ≥10%). Few POAI patients were classified into the low–high group. These are reported for completeness but not further analysed as their apparent response is probably due to measurement variability around the dichotomisation cut-point. Post-hoc subgroup analyses explored associations between Ki67 and disease outcome by chemotherapy use and age with a view to avoid confounding of interpretation. In addition to the predefined 10% Ki67 dichotomisation, chosen to ensure consistency with other neoadjuvant trials,[12,14] other cut-points were explored using Harrell's C coefficient[19] including that for complete cell cycle arrest (CCCA; Ki67 ≤2·7%[20]).

Previous analyses[21] of change in Ki67 in 679 control group patients with paired samples available indicated that in patients with a core-cut surgery sample the median proportional reduction was –4·1% (IQR –27·8 to 34·8), whereas in those with a resection sample at surgery, the median proportional reduction in Ki67 between baseline and surgery was –17·7% (IQR –44·2 to 12·7) in contrast with an earlier small pilot study.[16] From these findings, it was assumed that, for a given surgical sample, change in Ki67 score would be proportionally approximately 15% less if the sample was core-cut rather than resection (eg, 10% reduction with resection sample translated to 8·5% for core-cut). To account for this difference, Ki67 data and the analyses linking Ki67 and time to recurrence were done with $Ki67_{2W}$ corrected according to surgical sample type. $Ki67_{2W}$ scores from resection samples were increased proportionally by 15%. This correction factor was derived (and used) in control participants and similarly applied to participants in the POAI group. The correction was also made for patients for whom surgical sample type was unknown. For cases where $Ki67_{2W}$ was 0%, no adjustment was made.

This manuscript describes the primary endpoint analysis, time to recurrence after a 5-year median follow-up for both hypotheses; first by randomised POAI allocation and second exploring the ability of Ki67 to predict disease outcome. No formal interim analyses were planned or done before the primary analysis. For this purpose, a database snapshot was taken on Aug 8, 2017 for data presented at the San Antonio Breast Cancer Conference 2017 and updated with a second database snapshot taken on Feb 6, 2018. All analyses were done by means of Stata (version 13.1). A p value of less than 0·05 was deemed to be significant.

This study is registered with ClinicalTrials.gov, NCT02338310; the European Clinical Trials database, EudraCT2007-003877-21; and the ISRCTN registry, ISRCTN63882543.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Oct 13, 2008, and April 16, 2014, 4486 patients were entered from 130 UK centres. Six patients subsequently withdrew consent for data to be used and therefore 4480 patients (2976 POAI, 1504 control) were included in the modified intention-to-treat analysis (figure 1).

Median age at randomisation was 67·1 years (IQR 61·5–74·8), 2536 (57%) of 4480 patients had a tumour size up to 2 cm, and all but eight (<1%) patients were confirmed locally to have hormone receptor-positive tumours (table 1). 23 (1%) of 4480 patients did not have surgery as planned (16 patients in the POAI group and seven in the control group; figure 1). Adherence to trial

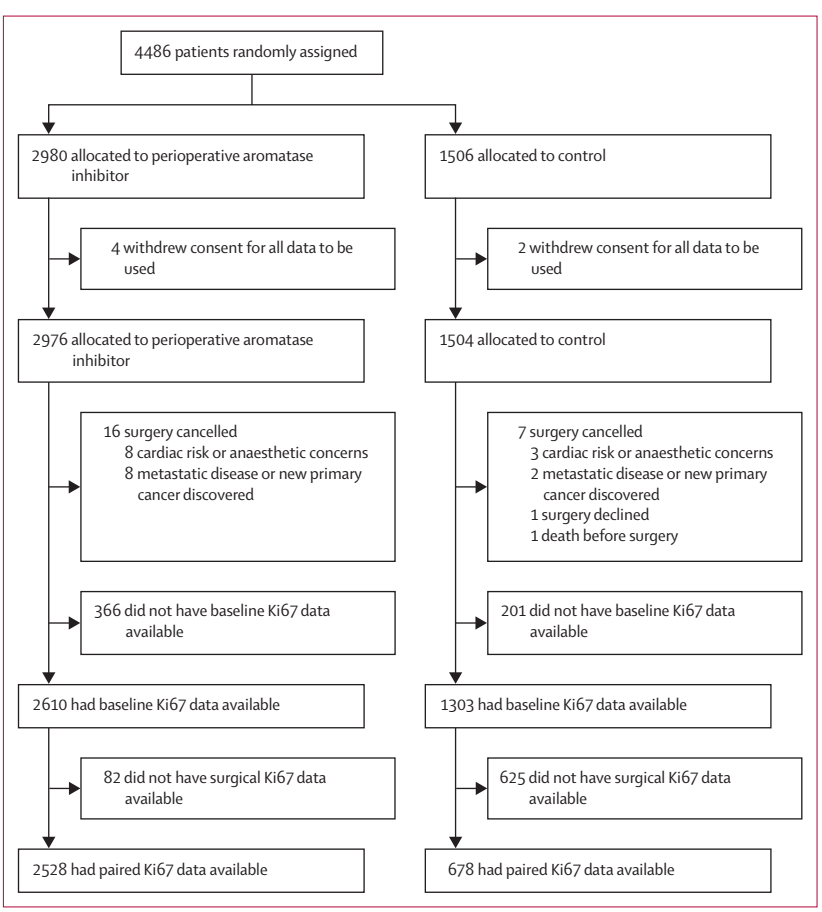

Figure 1: **Trial profile**

	Demographics at randomisation and tumour characteristics from the diagnostic core		Surgery details and tumour characteristics from surgery	
	Perioperative aromatase inhibitor group (n=2976)	Control group (n=1504)	Perioperative aromatase inhibitor group (n=2960)	Control group (n=1497)
Age group at randomisation, years				
<50	9 (<1%)	3 (<1%)	..	..
50–59	579 (19%)	291 (19%)	..	..
60–69	1245 (42%)	609 (40%)	..	..
70–79	808 (27%)	429 (29%)	..	..
≥80	335 (11%)	172 (11%)	..	..
Age, years	67·1 (61·5–74·9)	67·3 (61·5–74·8)	..	..
Planned aromatase inhibitor				
Anastrozole	954 (32%)	483 (32%)	..	..
Letrozole	2022 (68%)	1021 (68%)	..	..
Hormone receptor status				
Positive	2971 (100%)	1501 (100%)	..	..
Negative	4 (<1%)	1 (<1%)	..	..
Missing	1 (<1%)	2 (<1%)	..	..
HER2 status				
Positive	..	..	317 (11%)	152 (10%)
Negative	..	..	2606 (88%)	1316 (88%)
Unknown or missing	..	..	37 (1%)	29 (2%)
Hormone receptor and HER2 status*				
Hormone receptor-positive				
HER2 positive	...	..	317 (11%)	152 (10%)
HER2 negative	..	..	2606 (88%)	1316 (88%)
HER2 unknown	..	..	37 (1%)	29 (2%)
Hormone receptor-negative				
HER2 negative	..	..	4 (<1%)	1 (<1%)
Histological type				
Ductal†	2404 (81%)	1198 (80%)	2364 (80%)	1199 (80%)
Lobular	428 (14%)	224 (15%)	454 (15%)	236 (16%)
Special type‡§	105 (4%)	58 (4%)	124 (4%)	50 (3%)
Ductal carcinoma in situ or lobular carcinoma in situ¶	0	0	3 (<1%)	2 (<1%)
Not breast cancer‖	0	0	1 (<1%)	0
Not known	39 (1%)	24 (2%)	14 (<1%)	10 (1%)
Tumour grade				
G1	417 (14%)	234 (16%)	465 (16%)	184 (12%)
G2	1757 (59%)	843 (56%)	1968 (66%)	838 (56%)
G3	521 (18%)	279 (19%)	502 (17%)	463 (31%)
GX	0	1 (<1%)	..	..
Not known**	278 (9%)	145 (10%)	17 (1%)	6 (<1%)
Missing	3 (<1%)	2 (<1%)	8 (<1%)	6 (<1%)
Tumour size, cm††				
≤2	1666 (56%)	870 (60%)	1372 (46%)	671 (45%)
>2–5	1238 (42%)	599 (40%)	1448 (49%)	745 (50%)
>5	54 (2%)	28 (2%)	129 (4%)	74 (5%)
Missing	18 (1%)	7 (<1%)	11 (<1%)	7 (<1%)

(Table 1 continues on next page)

treatment and timelines are shown in the appendix (p 14). 177 (6%) of 2976 patients did not have the protocol defined duration of POAI (preoperatively <10 days or >21 days, postoperatively <10 days). The most common reasons were 63 (2%) had their surgery changed, 35 (1%) had less owing to adverse events (16 were in the presurgical period), and 30 (1%) had less owing to patient choice or omission. Surgical details and postsurgery tumour characteristics were well balanced between groups with the exception of pathological tumour grade, which was higher in the control group (p<0·0001; table 1). Adjuvant radiotherapy and anti-HER2 therapy were given after surgery with similar frequency for the two groups and in line with UK standard of care. Adjuvant chemotherapy was given to 770 (26%) of 2957 patients in the POAI group and 460 (31%) of 1493 patients in the control group (appendix p 15) with multivariable analyses attributing this to differences observed in postsurgical grade (appendix p 16). Following surgery, most (POAI 2507 [86%] of 2960 patients; control 1186 [81%] of 1497 patients) women were prescribed aromatase inhibitor monotherapy (appendix p 17).

With 62·9 months' median follow-up (IQR 58·1–74·1), 434 (10%) of 4480 women had a breast cancer recurrence (POAI 280 [9%] of 2976 patients, control 154 [10%] of 1504 patients; table 2) with no significant difference observed between the treatment groups (HR 0·92, 95% CI 0·75–1·12; p=0·40, adjusted HR 0·96, 0·77–1·19; p=0·70; figure 2A) with the proportion free from breast cancer recurrence at 5 years of 91·0% (89·9–92·0) in the POAI group and 90·4% (88·7–91·9) in the control group. Subgroup analyses according to clinical characteristics, including nodal status, were consistent with the overall effect (appendix p 2). Likewise, no significant differences between treatment groups were observed for relapse-free survival, time to local recurrence, and time to distant recurrence (table 3). Second breast cancer primaries developed in 26 (<1%) of 2976 women in the POAI group compared with 24 (2%) of 1504 in the control group. 561 patients had died (POAI 365 [12%] of 2976; control 196 [13%] of 1504). Almost half of deaths were attributable to a non-breast cancer cause; none were treatment related (table 2). There was no difference in overall survival between treatment groups. 5-year overall survival was 88·9% (95% CI 87·7–90·1) in the POAI group versus 88·9% (87·2–90·5) in the control group (unadjusted HR 0·94, 95% CI 0·79–1·12; p=0·50, adjusted HR 0·91, 0·75–1·10; p=0·33; figure 2B).

Selected menopausal symptoms were assessed in 4201 (94%) of 4480 women, with higher symptom rates observed for POAI (appendix p 18). The most commonly reported grade 3 adverse events were hot flushes (20 [1%] of 2801 patients in the POAI group *vs* six [<1%] of 1400 in the control group) and musculoskeletal pain (29 [1%] *vs* 13 [1%]). 11 patients each reported a single serious adverse reaction (appendix p 19); all in the POAI group. The most

common were pulmonary embolism (n=3) and musculoskeletal pain (n=3).

3913 (87%) of 4480 participants had $Ki67_B$ data available. 2528 (85%) of 2976 patients in the POAI group and 678 (45%) of 1504 in the control group had paired $Ki67_B$ and $Ki67_{2W}$ data available (figure 1). In 2316 (72%) of 3206 participants with paired Ki67 data, the surgical sample was a resection (1834 [73%] of 2528 patients in the POAI group; 474 [70%] of 678 patients in the control group) or the surgical sample type was unknown (six in the POAI group and two in the control group) and the $Ki67_{2W}$ scores for these resections and unknown surgical sample types were corrected as described. 688 (27%) of 2528 POAI and 202 (30%) of 678 control patients' surgical sample type was core-cut biopsy.

The median $Ki67_B$ score in the 3913 of 4480 patients with a sample available was 15·2% (IQR 8·6–26·0; POAI 15·3% [8·5–26·4]; control: 14·9% [8·6–25·1]). $Ki67_B$ values were different between HER2-negative and HER2-positive tumours (median 14·3% [IQR 8·2–24·6] in HER2-negative tumours, median 26·6% [17·0–37·4] in HER2-positive tumours; p<0·0001). After 2 weeks of POAI, Ki67 was significantly suppressed compared with little change in the control group. $Ki67_{2W}$ was markedly lower in the HER2-negative tumours compared with HER2-positive tumours (appendix p 3). In the control group, given the little overall change, $Ki67_{2W}$ was again lower in the HER2-negative tumours than in HER2-positive tumours (appendix p 3).

In patients with HER2-negative tumours in the POAI group (2235 of 2528 patients), 209 (9%) time to recurrence events were reported. For the time to recurrence endpoint, women with $Ki67_B$ less than 10% (732 [33%] of 2235 patients) had a better prognosis than those with a $Ki67_B$ of at least 10% (1503 [67%] of 2235 patients; appendix p 20). Women whose $Ki67_{2W}$ remained high (high–high group) were significantly more likely to have a recurrence than those whose $Ki67_{2W}$ had dropped below 10% (high–low group; unadjusted HR 2·59, 95% CI 1·93–3·47; p<0·0001, adjusted HR 2·10, 1·48–2·98; p<0·0001; figure 3A). Within the POAI-treated HER2-negative subpopulation, 5-year recurrence risk in women with low $Ki67_B$ and $Ki67_{2W}$ (low–low) was 4·3% (95% CI 2·9–6·3), 8·4% (6·8–10·5) with high $Ki67_B$ and low $Ki67_{2W}$ (high–low) and 21·5% (17·1–27·0) with high $Ki67_B$ and $Ki67_{2W}$ (high–high). Within the POAI-treated HER2-positive subpopulation, 5-year recurrence risk in the low–low group was 10·1% (95% CI 3·2–31·3), 7·7% (3·4–17·5) in the high-low group, and 15·7% (10·1–24·4) in the high–high group. Adding a high versus low classification at 2 weeks segregated groups in relation to their baseline Ki67 (appendix p 21).

The HER2-negative POAI-treated subpopulation post-hoc exploratory analyses relating to the combined effects of age and chemotherapy use suggested that in patients with $Ki67_B$ of at least 10%, who did not receive adjuvant chemotherapy, the residual $Ki67_{2W}$ (high or low) conferred a differential effect on prognosis as assessed by time to recurrence for both those aged less than 70 years and aged at least 70 years (appendix pp 4–9). Numbers were too small to fully define effects for the corresponding group (ie, $Ki67_B$ ≥10%) who did receive chemotherapy.

In patients with HER2-negative breast cancer in the control group, 56 time to recurrence events were reported in the 597 of 678 patients for whom $Ki67_{2W}$ was available. There was no difference in time to recurrence between the high–high and high–low groups (appendix pp 10, 22).

	Demographics at randomisation and tumour characteristics from the diagnostic core		Surgery details and tumour characteristics from surgery	
	Perioperative aromatase inhibitor group (n=2976)	Control group (n=1504)	Perioperative aromatase inhibitor group (n=2960)	Control group (n=1497)
(Continued from previous page)				
Definitive breast surgery				
Mastectomy	..	..	1051 (36%)	503 (34%)
Conservative surgery	..	..	1902 (64%)	992 (66%)
Missing	..	..	7 (<1%)	2 (<1%)
Definitive axillary surgery				
Yes	..	..	2911 (98%)	1470 (98%)
Clearance	..	..	916 (31%)	468 (31%)
Sampling	..	..	287 (10%)	150 (10%)
Sentinal lymph node biopsy	..	..	1708 (58%)	852 (57%)
No	..	..	42 (1%)	25 (2%)
Missing	..	..	7 (<1%)	2 (<1%)
Nodal status				
N0	..	..	1815 (61%)	892 (60%)
N1–3	..	..	801 (27%)	434 (29%)
N4+	..	..	334 (11%)	165 (11%)
Missing	..	..	10 (<1%)	6 (<1%)
Vascular invasion				
Yes	..	..	813 (27%)	445 (30%)
No	..	..	1990 (67%)	981 (66%)
Not reported	..	..	143 (5%)	63 (4%)
Missing	..	..	14 (<1%)	8 (1%)
Multi-focal disease				
Yes	..	..	381 (13%)	223 (15%)
No	..	..	2563 (87%)	1266 (85%)
Missing	..	..	16 (1%)	8 (1%)

Data are n (%) and median (IQR). Surgery details exclude patients for whom surgery was permanently cancelled. *One patient (perioperative aromatase inhibitor) with hormone receptor status unknown was HER2 negative; the remaining two patients (control) with hormone receptor status unknown also had HER2 status unknown. †Ductal includes patients with mixed ductal and lobular tumours. ‡Special types on the diagnostic core include mucinous, papillary, tubular, metaplastic carcinoma, microcapillary, anaplastic with basaloid nuclear pattern. §Special types from surgery specimen include mucinous, papillary, tubular, endocrine cell carcinoma, pure special type, metaplastic carcinoma clear cell, and basaloid, tubular, and cribiform carcinoma. ¶Presurgical histological types for these patients were coded as ductal carcinoma. ||Prehistological type was not known (this patient is recorded as ineligible). **Some UK hospitals do not routinely report grade on the diagnostic core. ††Presurgery this measurement is either by ultrasound or clinical examination. Patients are eligible if they have either a palpable tumour (clinical examination) of any size or a tumour with an ultrasound size of ≥1·5 cm. 618 patients had tumour size <1·5 cm, of which 607 had a tumour confirmed as palpable.

Table 1: **Baseline characteristics**

	Perioperative aromatase inhibitor group (n=2976)	Control group (n=1504)
Any disease-related first event		
Yes	541 (18%)	309 (21%)
No	2435 (82%)	1195 (80%)
Event contributing to primary endpoint (time to recurrence)		
Total	280 (9%)	154 (10%)
Local recurrence (isolated)	25 (1%)	13 (1%)
Distant recurrence*	236 (8%)	131 (9%)
Breast cancer death†	19 (1%)	10 (1%)
Other event		
Total	261 (9%)	155 (10%)
Breast second primary cancer	26 (1%)	24 (2%)
Non-breast second primary cancer	136 (5%)	80 (5%)
Intercurrent death	99 (3%)	51 (3%)
Deaths		
Total	365 (12%)	196 (13%)
Breast cancer	201 (7%)	110 (7%)
Other (intercurrent deaths)	164 (6%)	86 (6%)
Cardiovascular	41 (1%)	25 (2%)
Other cancer	59 (2%)	35 (2%)
Respiratory	37 (1%)	15 (1%)
Sepsis	14 (<1%)	5 (<1%)
Other‡	13 (<1%)	6 (<1%)

Data are n (%). If more than one first event was reported on the same date, it was included in the row here according to the following order of priority: distant recurrence, local recurrence, breast second primary cancer, non-breast second primary cancer, and intercurrent death. *Distant recurrence row included patients for whom distant recurrence is reported within 6 weeks of local recurrence. †Included 25 patients (18 perioperative aromatase inhibitor, seven in the control group) with unknown cause of death and no previous event; one patient had a second primary cancer before unknown cause of death and was not included here. ‡Other causes in the perioperative aromatase inhibitor group (n=13) were accident (n=2), acute kidney injury, Alzheimer's disease, ascending aortic aneurysm, haematemesis secondary to gastric ulcer, hepatic cirrhosis, multiorgan failure, myelofibrosis, old age with vascular deterioration and chronic kidney disease, portal hypertension, a fall, ascites, evidence of cirrhosis, postoperative complications relating to pituitary tumour operation, and renal failure; other causes in the control group (n=6) were complications post laparotomy, dementia, diabetes, meningioma, subdural haematoma, and suicide.

Table 2: **Disease-related first events and deaths**

A post-hoc sensitivity analysis in the HER2-negative subgroup combining the baseline data for POAI and control gave a 5-year recurrence risk of 4·7% (95% CI 3·5–6·3) for low Ki67B and 11·5% (95% CI 10·1–13·1) for high Ki67B (appendix p 24).

Prespecified exploratory analysis in the HER2-negative subgroup suggested an optimal cut-point around 15–20% for $Ki67_{B}$ and around 6–8% for $Ki67_{2W}$ and that using the CCCA threshold for $Ki67_{2W}$ had prognostic discrimination (appendix pp 11–13).

In patients with hormone receptor-positive, HER2-positive breast cancer in the POAI group (273 [10%] of 2528 patients), 33 time to recurrence events were reported. 143 women in the Ki67 high–high group had a recurrence compared with 94 in the high–low group, although the difference was not significant (unadjusted HR 2·08, 95% CI 0·88–4·90; p=0·093, adjusted HR 1·83, 0·71–4·73; p=0·21; figure 3B). Similar to the HER2-negative group, absolute risk of recurrence at 1, 3, and 5 years was higher in the high-high group than in the high–low group (appendix p 23). 5-year recurrence risk in the low-low group was 10·1% (95% CI 3·2–31·3), 7·7% (3·4–17·5) in the high–low group, and 15·7% (10·1–24·4) in the high–high group. In the 70 women with HER2-positive breast cancer in the control group, nine time to recurrence events were reported.

Discussion

POETIC is, to our knowledge, the largest trial of its kind to assess the potential of POAI therapy in patients with postmenopausal, hormone receptor-positive early breast cancer and it did not show any significant long-term improvement in disease outcomes with this approach. This was despite preclinical experimental evidence in a mouse model suggesting the contrary.[3,4] A smaller phase 3 clinical trial, which reported after POETIC was initiated, randomly assigned operable breast cancer patients (n=976, 50% hormone receptor-positive, 45% hormone receptor-negative, and 5% hormone receptor unknown) to surgery or an intramuscular injection of depot hydroxyprogesterone 500 mg 5–14 days before surgery; no significant benefit was observed in the overall population (HR 0·87, 95% CI 0·68–1·09; p=0·23), but the results suggested a hypothesis-generating potential disease-free survival improvement in node-positive subgroups (HR 0·72, 0·54–0·97; p=0·02).[22] In contrast, consistent with the overall finding, POETIC showed no suggestion of long-term outcome improvement with POAI overall or in the node-positive subgroup.

In POETIC, the frequency of chemotherapy was slightly lower in patients in the POAI group than in those in the control group. Multivariable regression supported the suggestion that this was probably because of multidisciplinary teams being influenced by pathological tumour grade, which was on average lower in the patients in the POAI group. This absolute difference was small however (5%), and since the overall event rate was less than 20% would have had an imperceptible effect on outcome comparisons.

On a pragmatic note, it is common practice to start some patients on preoperative endocrine therapy if there has to be a significant delay in surgery for any reason. Despite not showing any statistical evidence of clinical benefit, our results provide reassurance that there is no detriment to this practice.

The second aim of this trial was to explore whether the measurement of tumour Ki67 2 weeks after starting treatment could predict disease outcome better than baseline Ki67 alone, thus providing the basis of a simple and inexpensive test to personalise adjuvant treatment in patients with hormone receptor-positive, HER2-negative breast cancer. Previously, IMPACT had shown

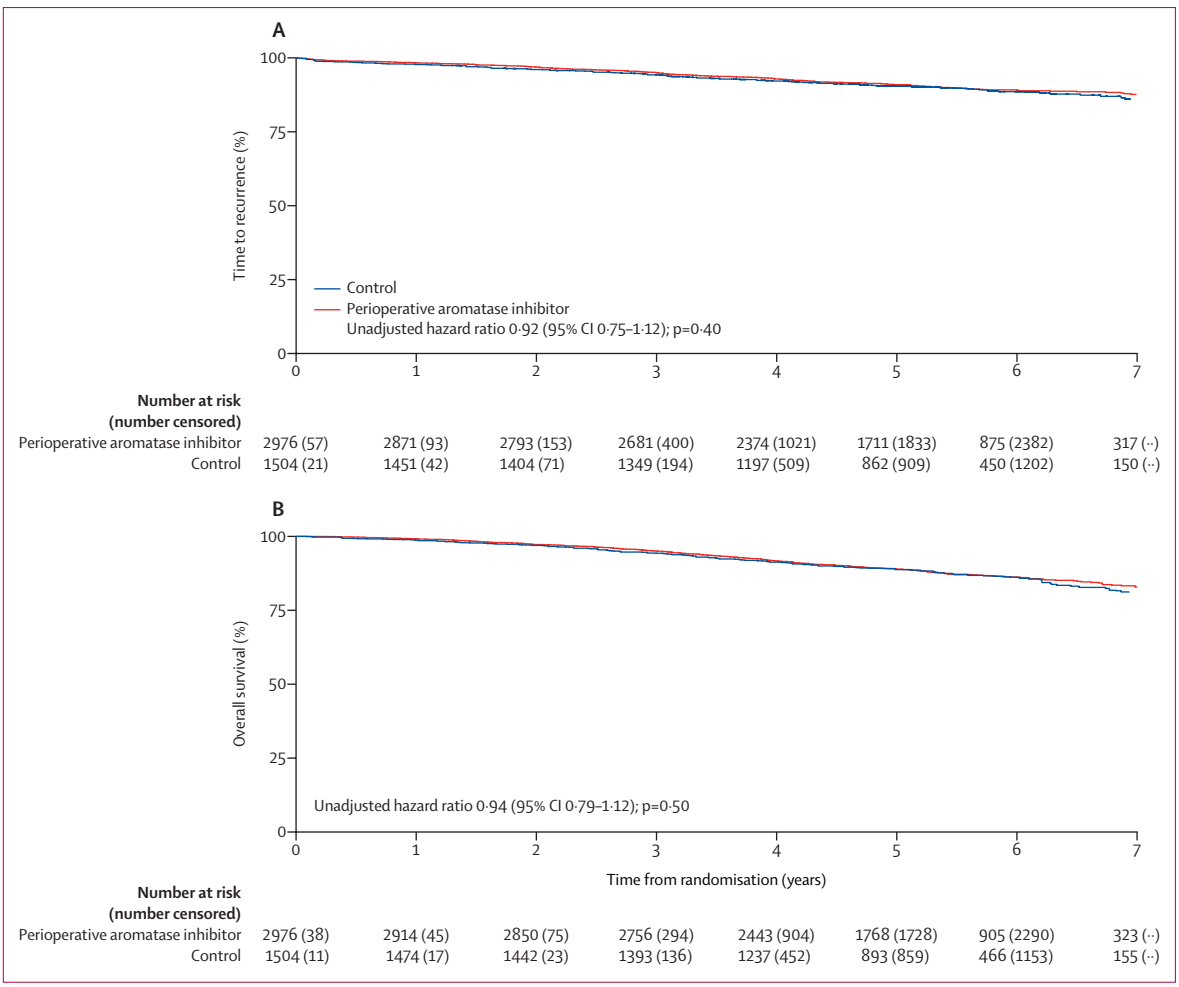

Figure 2: **Kaplan-Meier survival curve by randomised treatment group for time to recurrence (A) and overall survival (B)**
In part A test for proportionality, p=0·58. In part B test for proportionality, p=0·82.

that 2-week on-treatment Ki67 predicted outcome better than baseline and, unlike baseline, was significant in multivariable analysis.[12] POETIC has provided evidence for the clinical validity of on-treatment aromatase inhibitor $Ki67_{2W}$ in addition to $Ki67_{B}$ to predict those with high residual risk of recurrence in spite of standard-of-care therapy. At the initiation of POETIC, we believed that the evidence was insufficient to withhold or direct therapy on the basis of the $Ki67_{2W}$. Our results provide an early indication of endocrine sensitivity or resistance including for the large number of patients who are not routinely considered for adjuvant chemotherapy.

Separate clearly defined adjuvant treatment pathways for HER2-positive and HER2-negative breast cancers now exist and we therefore analysed these groups separately when considering prognostic risk. The HER2-positive subgroup was small with relatively few events. Focus for exploratory analysis was therefore on the HER2-negative subgroup, which comprised approximately 90% of the POETIC population.

	Number of events		Unadjusted hazard ratio	Adjusted hazard ratio	5-year survival estimate	
	Perioperative aromatase inhibitor group	Control group			Perioperative aromatase inhibitor group	Control group
Relapse-free survival	385 (13%)	207 (14%)	0·94 (0·79–1·11); 0·47	0·95 (0·79–1·14); 0·59	87·9% (86·6–89·1)	87·6% (85·7–89·2)
Time to local recurrence	41 (1%)	24 (2%)	0·86 (0·52–1·43); 0·57	0·92 (0·54–1·56); 0·75	98·6% (98·1–99·0)	98·5% (97·6–99·0)
Time to distant recurrence	262 (9%)	147 (10%)	0·90 (0·73–1·10); 0·30	0·94 (0·75–1·18); 0·59	91·7% (90·5–92·6)	90·9% (89·2–92·3)

Data are n (%), hazard ratio (95% CI); p value, and % (95% CI). Models adjusted for progesterone receptor status (positive, negative, unknown), HER2 status (positive, negative, unknown), presurgical tumour grade (G1, G2, and G3), pathological tumour size (continuous), presurgical histological type (ductal, lobular, special type), nodal status (N0, N1–3, and N4+), age at randomisation (continuous), and vascular invasion (yes, no). Test for proportionality for relapse-free survival, p=0·69; for time to local recurrence, p=0·97, and for time to distant recurrence, p=0·52.

Table 3: **Summary of disease-related endpoints**

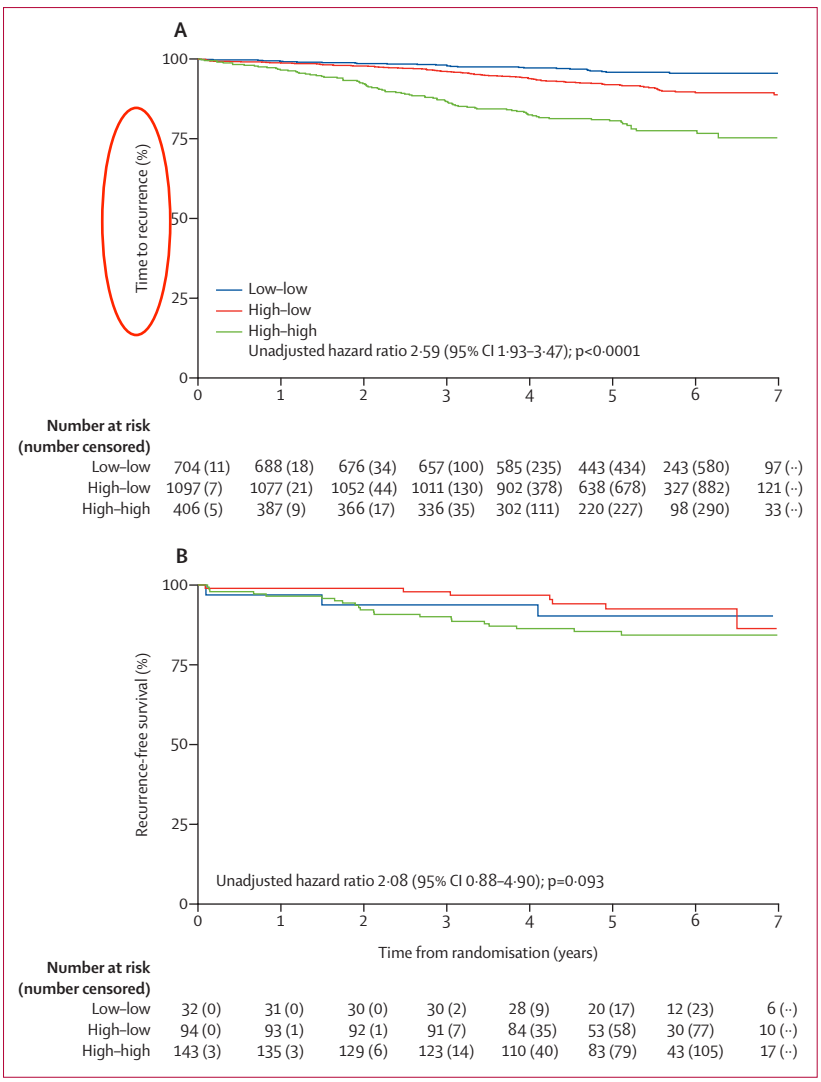

Figure 3: **Kaplan-Meier survival curve for time to recurrence by $Ki67_B$ and $Ki67_{2W}$ for patients with hormone receptor-positive and HER2-negative breast cancer (A) and hormone receptor-positive, HER2-positive breast cancer (B) in the perioperative aromatase inhibitor group**

Low–low=$Ki67_B$ and $Ki67_{2W}$ <10%. High–low=$Ki67_B$ ≥10% and $Ki67_{2W}$ <10%. High–high=$Ki67_B$ and $Ki67_{2W}$ ≥10%. B=baseline. 2w=2weeks. 28 patients with hormone receptor-positive breast cancer and HER2-negative breast cancer and four patients with hormone receptor-positive and HER2-positive breast cancer in the low-high group were omitted from the figure.

Previously, it had been shown that patients with a low $Ki67_B$ have a better prognosis than those with a high $Ki67_B$ value.[23] POETIC confirmed this in a larger prospective population, dichotomising $Ki67_B$ at 10% with 5-year recurrence risk in HER2-negative patients in the POAI group of 4·4% for low $Ki67_B$ and 11·8% for high $Ki67_B$. To our knowledge, this is the first large published dataset that makes use of the Ki67 scoring methodology recommended by the International Ki67 in Breast Cancer Working Group; the strong association of Ki67 at baseline with prognosis served as a clinical validation of that methodology.[15] Patients whose $Ki67_B$ was low did well on standard of care, with approximately 85% of those receiving endocrine therapy alone. It could be the case that if the patient's clinical pathological features led to chemotherapy being given, this approach might have contributed to the good outcomes. But irrespective of adjuvant treatment, it is reasonable to conclude that $Ki67_{2W}$ did not add significant prognostic or predictive information in this subgroup.

In contrast, for patients whose tumours had a high baseline Ki67 in the POAI group, 73% had a low $Ki67_{2W}$ 2 weeks after starting treatment; those patients had a better prognosis at 5 years than those who continued to have a high $Ki67_{2W}$ (8·4% *vs* 21·5% 5-year recurrence risk). To what extent could this observation be applied to clinical practice?

The answer to this question is influenced by the limitations of this trial. The first concerns the optimal cutoff for Ki67, and we have shown that dichotomising for cutoffs other than 10% merit further exploration. The second limitation concerns interpreting the data in relation to age and chemotherapy usage. Older age has already been shown to be an independent prognostic factor in breast cancer[24] and POETIC patients aged at least 70 years had poorer outcomes than those aged below 70 years. Since a substantial minority (26%) of POAI patients had adjuvant chemotherapy, this could be a potential confounding factor in the interpretation of $Ki67_{2W}$ in relation to prognosis and prediction of the value of endocrine therapy alone. To address this, we repeated our analyses in patients according to their receipt of adjuvant chemotherapy. This confirmed a persisting worse outcome for tumours high–high after 2 weeks of an aromatase inhibitor compared with high–low in the 74% of patients not receiving chemotherapy. In the corresponding groups who received chemotherapy, numbers were insufficient to determine a prognostic Ki67 effect or to define a plausible beneficial chemotherapy effect.

In the two-thirds of patients below the age of 70 years not receiving chemotherapy, the overall outcome in terms of recurrence risk was better, probably reflecting the choice of omitting chemotherapy for better prognosis patients. But the key point was that in this population of patients non-confounded by chemotherapy, 21% with high $Ki67_B$ remained high at surgery (high–high) and those had 11·2% 5-year recurrence risk (arguably meriting chemotherapy in addition), compared with the low–low groups in which recurrence by 5 years was only 1·6% and the high–low group in which recurrence by 5 years was only 2·9% (indicating that additional chemotherapy would be of no clinically relevant benefit). This exploratory outcome must be interpreted with caution but further supports the prognostic value of measuring Ki67 at 2 weeks.

Similar findings were observed for patients aged at least 70 years. Only 59 of those patients received chemotherapy, too few to provide statistical confidence in the relationship between Ki67 and outcome. In those

aged at least 70 years who did not receive chemotherapy, there was again a large difference in outcome between the high–low and high–high groups (5-year recurrence risk 12·3% *vs* 34·5%), again supporting the discriminatory power of measuring Ki67 at 2 weeks, even though the absolute risks were greater.

The prespecified $Ki67_{2w}$ 10% cut-point was chosen for consistency with ongoing clinical trials (ALTERNATE [NCT01953588]; ADAPT [NCT01779206]). The relationship of $Ki67_{2w}$ with recurrence risk is continuous and as illustrated by our analysis by means of CCCA, other cut-points might be selected if appropriate for a specific use (eg, assessing the value of well-tolerated additional treatment).

In conclusion, in POETIC, giving perioperative endocrine therapy with an aromatase inhibitor had no significant effect on long-term outcome. The trial also showed that using $Ki67_{B}$ and aromatase inhibitor on-treatment $Ki67_{2w}$ could help guide adjuvant treatment decisions. First, we believe that we have identified a subgroup with a low baseline Ki67 who have a sufficiently good prognosis that the majority will do well on standard endocrine therapy alone (except perhaps for a minority as dictated by other clinical–pathological factors) and who do not require a repeat 2-week biopsy. Second, giving POAI to the subgroup with high baseline Ki67 can differentiate two groups of patients according to their 2-week Ki67 value: those who convert to a low Ki67 might not need anything beyond adjuvant endocrine therapy (taking consideration of other clinical-pathological factors), whereas those with a high Ki67 that has remained high, should be considered for further adjuvant treatments and trials. There are, of course, now several commercially available genomic platforms developed to provide the same kind of prognostic and predictive information for the individual patient.[8,9] But these tests are expensive, they often involve central testing of tissue, which has to be sent long distances with inevitable time delay, and results can differ between the platforms. Ki67 as used in POETIC potentially offers an inexpensive, easy and quick alternative in situations in which genomic testing is not readily available.

Contributors

IS was chief investigator. IS and JR assisted with trial design, protocol development, participant recruitment, data collection, data interpretation, and writing and were Trial Management Group members. LK assisted with statistical analysis, data interpretation, and writing and was a Trial Management Group member. MW was a patient advocate and a Trial Management Group member. AE, CH, KH, MS, AS, and RV assisted with participant recruitment and data collection and were Trial Management Group members. CK and EM assisted with data collection, data analysis, and data interpretation, and were Trial Management Group members. MC assisted with data analysis and data interpretation, and was a Trial Management Group member. JB assisted with trial management, data collection, and data management, and was a Trial Management Group member. JM assisted with trial design, protocol development, statistical analysis, and data interpretation, and was a Trial Management Group member. KS assisted with data collection. AD assisted with data analysis and data interpretation. JMB assisted with trial design, protocol development, statistical analysis, data interpretation, and writing and was a Trial Management Group member. MD assisted with trial design, protocol development, data analysis, data interpretation, and writing, and was a Trial Management Group member. All authors reviewed the manuscript before submission.

Declaration of interests

MD reports grants from Cancer Research UK, during the conduct of the study; and personal fees from Radius, Roche, Myriad, Orion, G1 Therapeutics, Nanostring, AbbVie, H3 Biomedicine, Lilly, and the ICR Rewards for Inventors Scheme, outside the submitted work. JMB reports grants from Cancer Research UK, during the conduct of the study; grants from Medivation; grants and non-financial support from AstraZeneca, Merck Sharp & Dohme, Puma Biotechnology, Clovis Oncology, Pfizer, Janssen-Cilag, Novartis, and Roche, outside the submitted work. LK reports grants from Cancer Research UK, during the conduct of the study. All other authors declare no competing interests.

Data sharing

De-identified data will be made available to other researchers on request, subject to approval of a formal data access request in accordance with the ICR-CTSU data and sample access policy. Trial documentation including the protocol are available on request by contacting poetic-icrctsu@icr.ac.uk. The ICR-CTSU supports the wider dissemination of information from the research it does, and increased cooperation between investigators. Trial data is collected, managed, stored, shared, and archived according to ICR-CTSU Standard Operating Procedures in order to ensure the enduring quality, integrity, and utility of the data. Formal requests for data sharing are considered in line with the Institute of Cancer Research Clinical Trials and Statistics Unit (ICR-CTSU) procedures with due regard given to funder and sponsor guidelines. Requests are via a standard proforma describing the nature of the proposed research and extent of data requirements. Data recipients are required to enter a formal data sharing agreement which describes the conditions for release and requirements for data transfer, storage, archiving, publication and intellectual property. Requests are reviewed by the Trial Management Group (TMG) in terms of scientific merit and ethical considerations including patient consent. Data sharing is allowed if proposed projects have a sound scientific or patient benefit rationale as agreed by the TMG and approved by the Trial Steering Committee as required. Restrictions relating to patient confidentiality and consent will be limited by aggregating and anonymising identifiable patient data. Additionally all indirect identifiers that might lead to deductive disclosures will be removed in line with Cancer Research UK Data Sharing Guidelines. Additional documents might be shared if approved by the TMG and Trial Steering Committee (eg, statistical analysis plan and informed consent form).

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Smith I, Robertson J, Kilburn L, et al. Long-term outcome and prognostic value of Ki67 after perioperative endocrine therapy in postmenopausal women with hormone-sensitive early breast cancer (POETIC): an open-label, multicentre, parallel-group, randomised, phase 3 trial. *Lancet Oncol* 2020; **21:** 1443–54.

Long-term outcome and prognostic value of Ki67 after perioperative endocrine therapy in postmenopausal women with hormone sensitive early breast cancer (POETIC): an open-label, multicentre, parallel-group, randomised, phase 3 trial

Supplementary web-appendix

Additional details on study design and participants

Eligibility:
Exclusion criteria included previous invasive breast cancer (except surgically treated DCIS or LCIS); concurrent bilateral breast cancer; multiple unilateral tumours with different ER/PR/HER2 status, grade or histological type; concurrent use (<4 weeks of diagnostic tissue taken) of HRT or any other oestrogen-containing medication (including vaginal oestrogens); any previous use of oestrogen implants; prior endocrine therapy or chemotherapy for breast cancer; any invasive malignancy diagnosed within previous 5 years (other than basal cell carcinoma or cervical carcinoma in situ); any severe co-incident medical disease; treatment with an unlicensed or investigational drug within previous 4 weeks and current, continuous, long-term systemic steroid usage.

Post-menopausal status was defined as a woman aged ≥50 years fulfilling any one of the following criteria:

i) with amenorrhoea >12 months and an intact uterus;
ii) has undergone a bilateral oophorectomy;
iii) in women who have undergone a hysterectomy, then FSH levels within the postmenopausal range (utilising ranges from the testing laboratory facility) are required if the patient is aged <55 years; or
iv) in women who have been on HRT within the last 12 months and therefore not amenorrhoeic, FSH levels within the postmenopausal range (utilising ranges from the testing laboratory facility) are required if the patient is aged <55 years.

Study oversight:
The Institute of Cancer Research Clinical Trials & Statistics Unit, (ICR-CTSU) had responsibility for trial coordination, data management and all statistical analyses including central monitoring, interim and final analysis. Safety and efficacy data were reviewed regularly by an Independent Data Monitoring Committee (IDMC) and trial oversight was provided by an independent Trial Steering Committee on behalf of the funder and sponsors. The Trial Management Group was responsible for the day-to-day running of the trial.

Procedures:
Investigators were encouraged to ensure that adjuvant treatment and participation in subsequent trials was not influenced by the POETIC treatment allocation. Surgery was breast conserving or mastectomy. With breast conservation, clear margins were required, via re-excision if necessary. Primary breast reconstruction and other oncoplastic procedures to improve cosmetic outcome were allowed. Initial axillary surgery included sentinel lymph node biopsy (SLNB) (or sampling if SLNB was not possible) or axillary clearance. If SLNB or sampling identified axillary node involvement, subsequent axillary node resection or axillary radiotherapy was to be undertaken. Post-operative radiotherapy was given where required. The protocol anticipated adjuvant endocrine therapy for at least five years. Adjuvant chemotherapy, and adjuvant trastuzumab for patients with HER2+ cancers, were to be given as required. Staging investigations and follow-up were conducted according to local practice with imaging and biochemical investigations carried out as clinically indicated where recurrence or other significant clinical problems were suspected.

Optional samples details
Optional tissue samples in RNA-later could also be provided at baseline, surgery and post-surgery follow-up.

In addition, upon recurrence and if patients consented and tissue available, an FFPE sample was collected from the site of recurrence.

Blood samples were taken prior to randomisation (one in EDTA and one in PAXgene), at surgery (one in EDTA), at the first follow-up post-surgery (one in EDTA) and at a late follow-up visit at any time-point two years or more from randomisation (one in EDTA and one in Streck).

Additional tables and figures

Figure A1: Forest plot of subgroup analyses for TTR endpoint

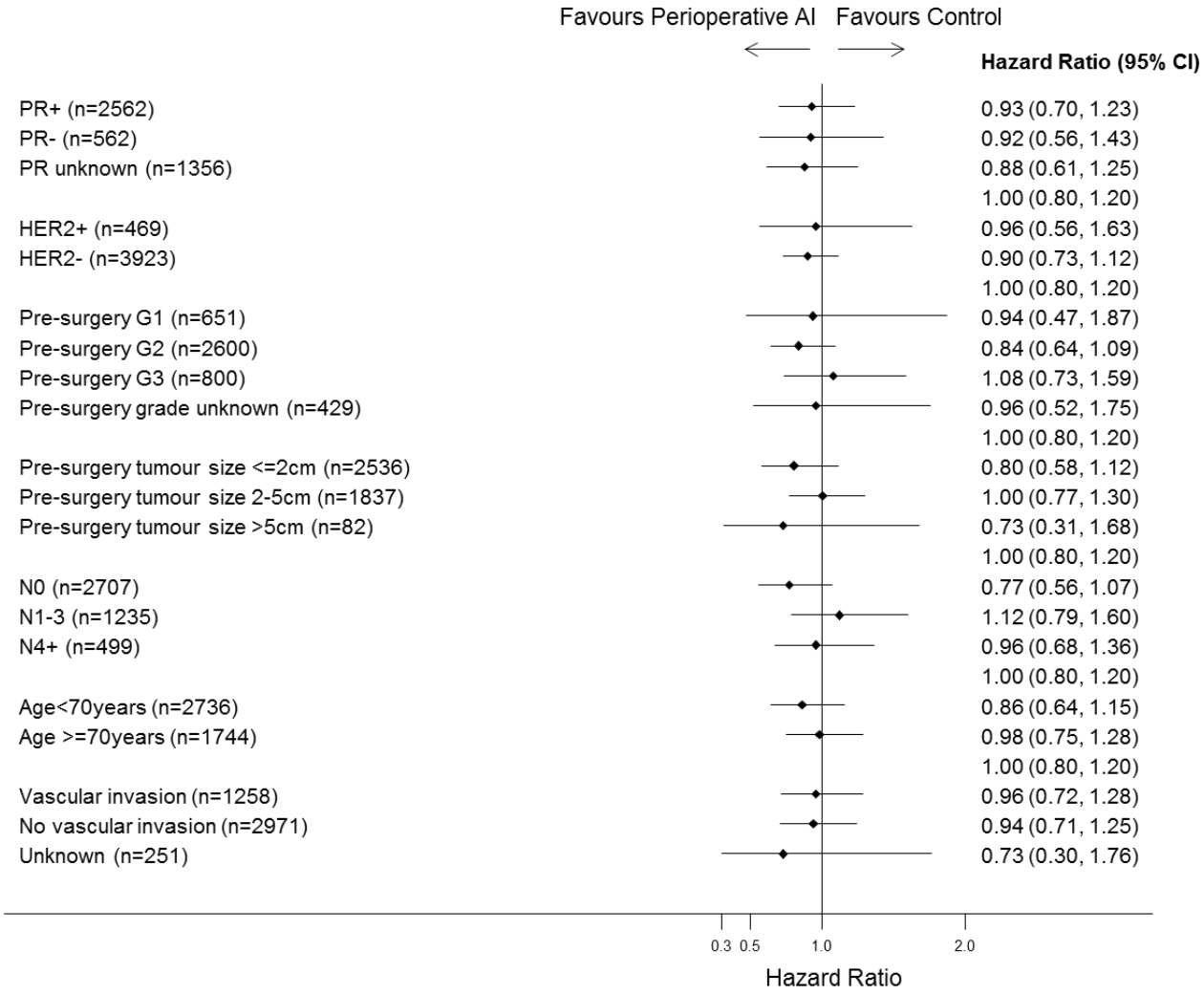
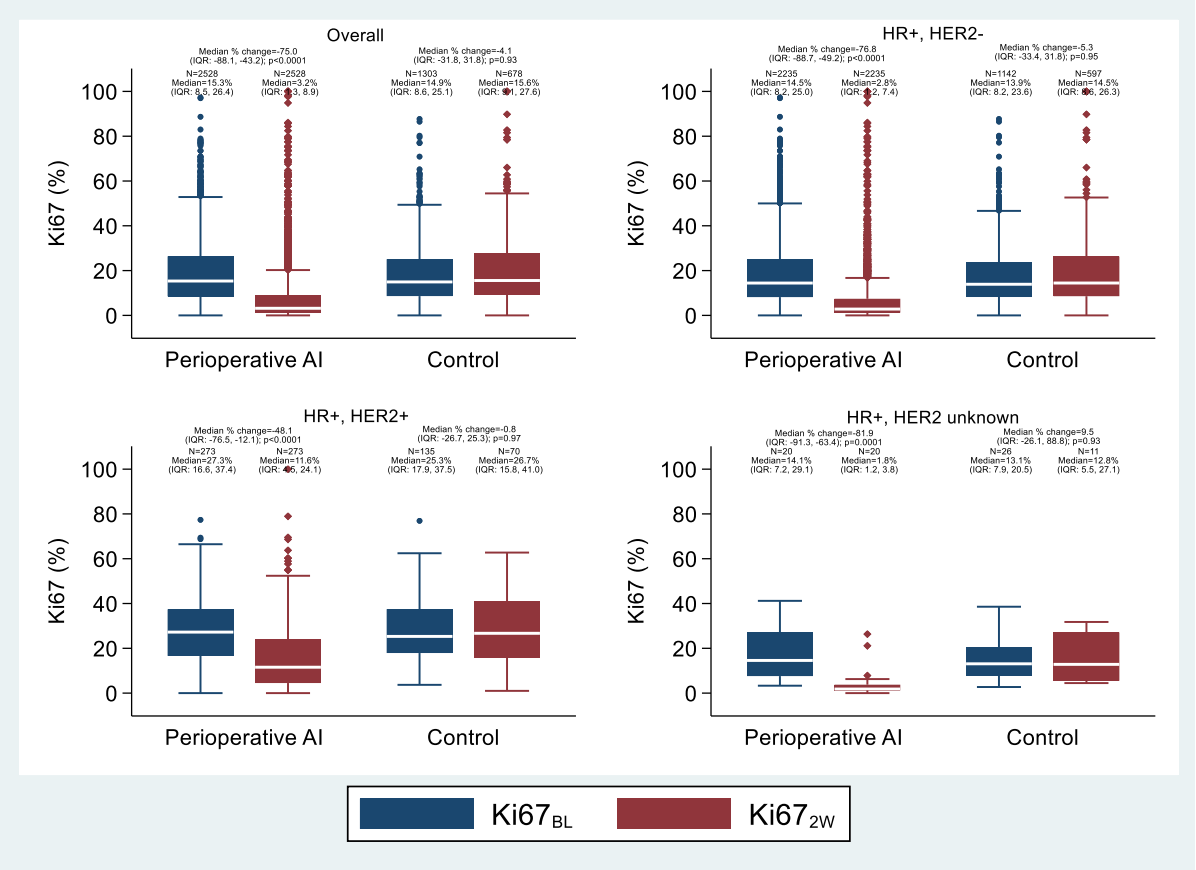
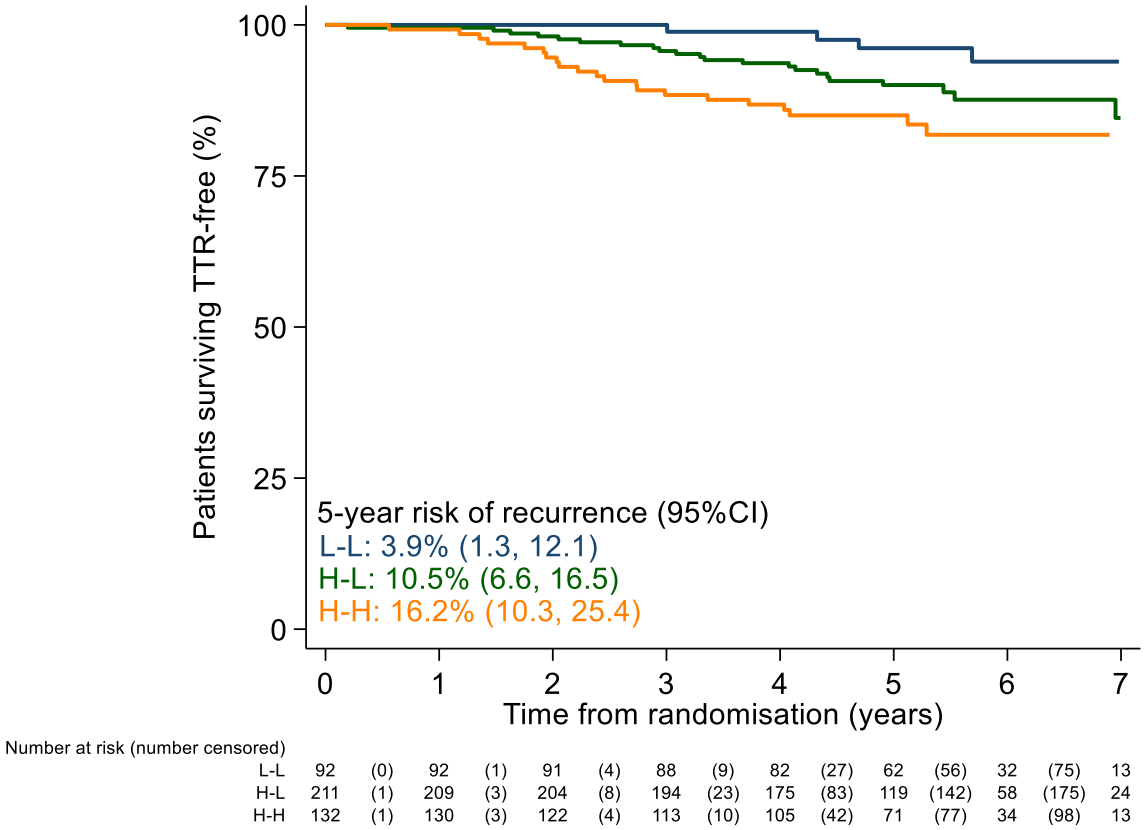


Figure A2: Median (IQR) ki67 (%) by time-point, treatment group and HER2 status



In the HER2-negative POAI-treated sub-population receiving chemotherapy, aged <70 years old with $Ki67_B \geq 10\%$ no evidence was observed of a difference in TTR between low and high $Ki67_{2W}$ (Figure A3a) although the numbers were small. In those <70 with $Ki67_B \geq 10\%$ not having chemotherapy there was a statistically significant difference in TTR between low and high $Ki67_{2W}$ (Figure A3b).

Figure A3a: Kaplan Meier survival curve for time to recurrence by $Ki67_B$ and $Ki67_{2W}$ for patients with HR+ and HER2-negative breast cancer allocated perioperative AI who are <70 years old and received chemotherapy (N=442)



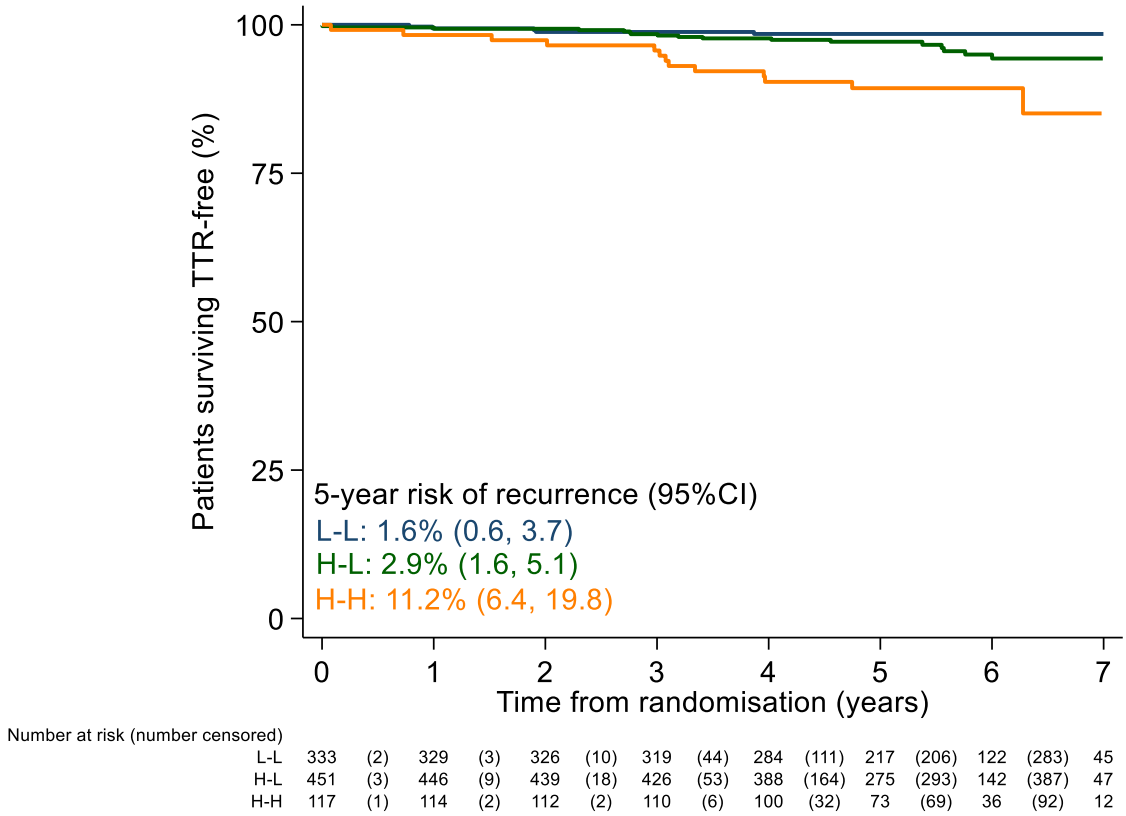
Number at risk (number censored)

L-L	92	(0)	92	(1)	91	(4)	88	(9)	82	(27)	62	(56)	32	(75)	13
H-L	211	(1)	209	(3)	204	(8)	194	(23)	175	(83)	119	(142)	58	(175)	24
H-H	132	(1)	130	(3)	122	(4)	113	(10)	105	(42)	71	(77)	34	(98)	13

Patients in the L-H group are omitted from the figure n=7 patients

In those with $Ki67_B \geq 10\%$ (n=343) unadjusted HR=1·55, 95%CI: 0·86, 2·81; p=0·15, adjusted HR=1·57, 95%CI: 0·79, 3·17; p=0·21

Figure A3b: Kaplan Meier survival curve for time to recurrence by $Ki67_B$ and $Ki67_{2W}$ for patients with HR+ and HER2-negative breast cancer allocated perioperative AI who are <70 years old and did not receive chemotherapy



Number at risk (number censored)

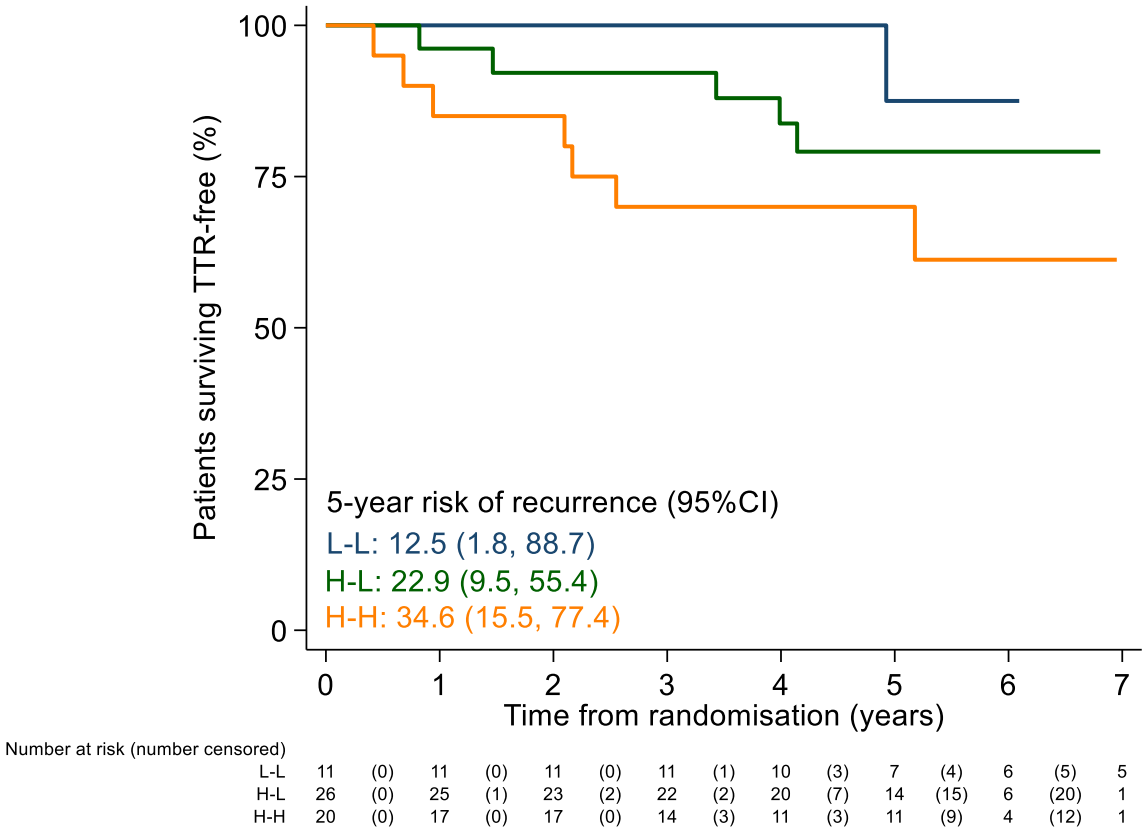
L-L	333	(2)	329	(3)	326	(10)	319	(44)	284	(111)	217	(206)	122	(283)	45
H-L	451	(3)	446	(9)	439	(18)	426	(53)	388	(164)	275	(293)	142	(387)	47
H-H	117	(1)	114	(2)	112	(2)	110	(6)	100	(32)	73	(69)	36	(92)	12

Patients in the L-H group are omitted from the figure n=12 patients

In those with $Ki67_B \geq 10\%$ (n=568) unadjusted HR=3·01, 95%CI: 1·46, 6·19; p=0·0028, adjusted HR=2·78, 95%CI: 1·20, 6·43; p=0·017

In the small number aged ≥70 years with $Ki67_B$≥10% who received chemotherapy there was clear but non-significant separation in TTR between low and high $Ki67_{2W}$ (Figure A3c). In those aged ≥70 with a $Ki67_B$≥10% not receiving chemotherapy there was a statistically significant difference in TTR between low and high $Ki67_{2W}$ (Figure A3d). Overall outcomes irrespective of chemotherapy for those <70 and ≥70 are also provided (Figures A4a and b).

Figure A3c: Kaplan Meier survival curve for time to recurrence by $Ki67_B$ and $Ki67_{2W}$ for patients with HR+ and HER2-negative breast cancer allocated perioperative AI who are ≥70 years old and received chemotherapy



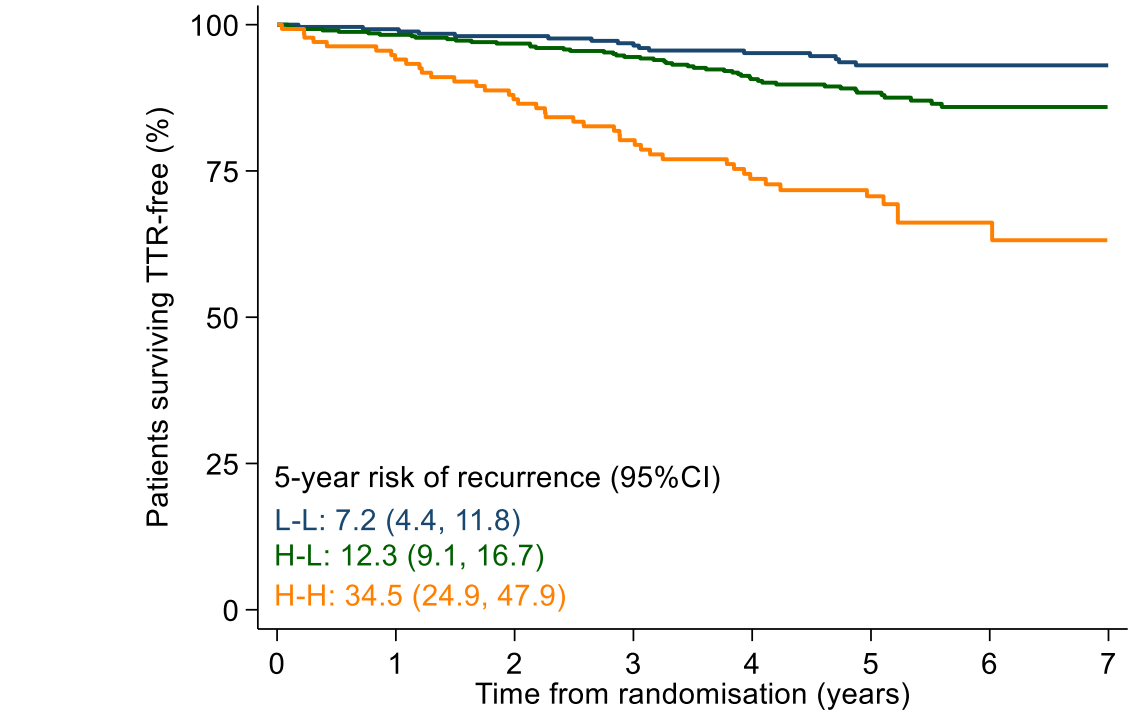
Number at risk (number censored)

L-L	11	(0)	11	(0)	11	(0)	11	(1)	10	(3)	7	(4)	6	(5)	5
H-L	26	(0)	25	(1)	23	(2)	22	(2)	20	(7)	14	(15)	6	(20)	1
H-H	20	(0)	17	(0)	17	(0)	14	(3)	11	(3)	11	(9)	4	(12)	1

Patients in the L-H group are omitted from the figure n=2 patients

In those with $Ki67_B$≥10% (n=46) unadjusted HR=2·09, 95%CI: 0·66, 6·59; p=0·21, adjusted HR=1·91, 95%CI: 0·17, 22·1; p=0·60

Figure A3d: Kaplan Meier survival curve for time to recurrence by $Ki67_B$ and $Ki67_{2W}$ for patients with HR+ and HER2-negative breast cancer allocated perioperative AI who are ≥70 years old and did not receive chemotherapy



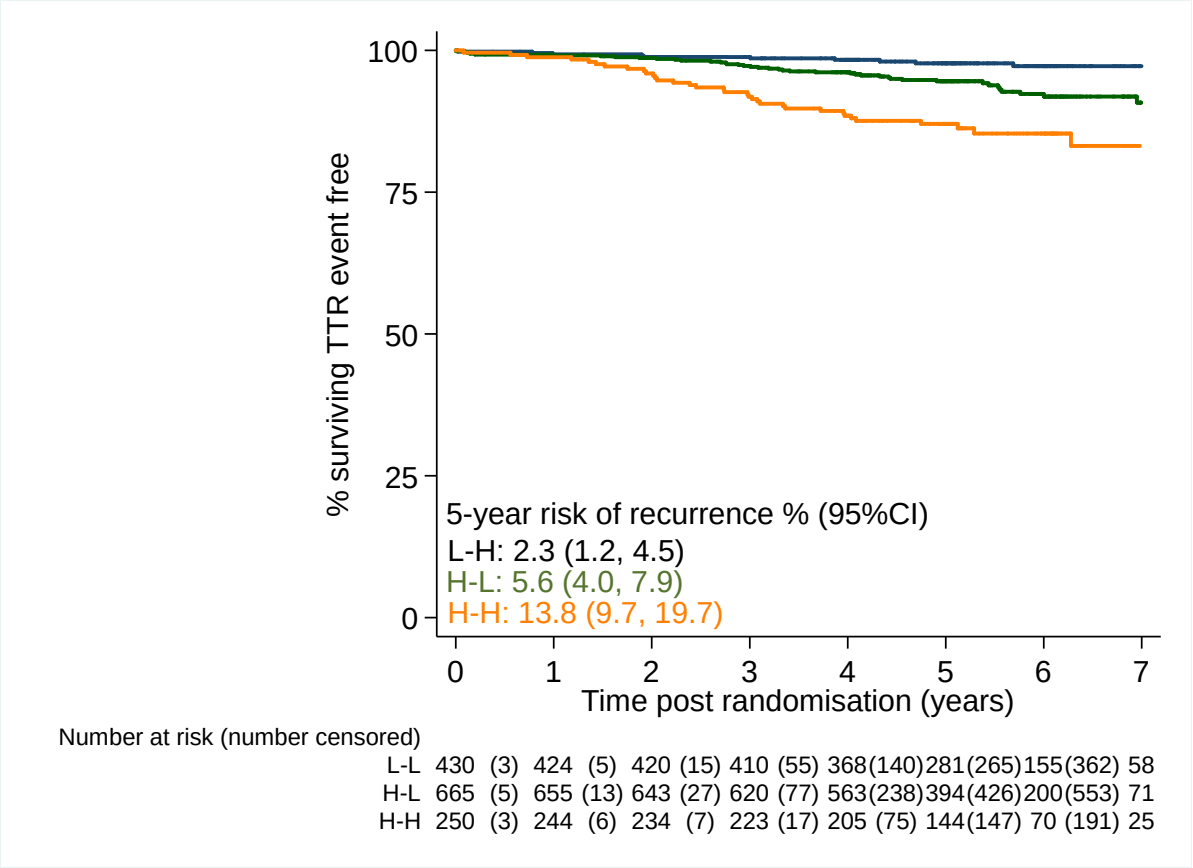
N at risk (events)

L-L	262	(7)	253	(12)	245	(18)	236	(43)	207	(91)	155	(164)	82	(212)	34
H-L	403	(1)	395	(6)	384	(14)	367	(50)	317	(132)	228	(234)	121	(306)	49
H-H	135	(2)	125	(3)	115	(10)	99	(15)	86	(33)	65	(71)	24	(87)	7

Patients in the L-H group are omitted from the figure n=7 patients

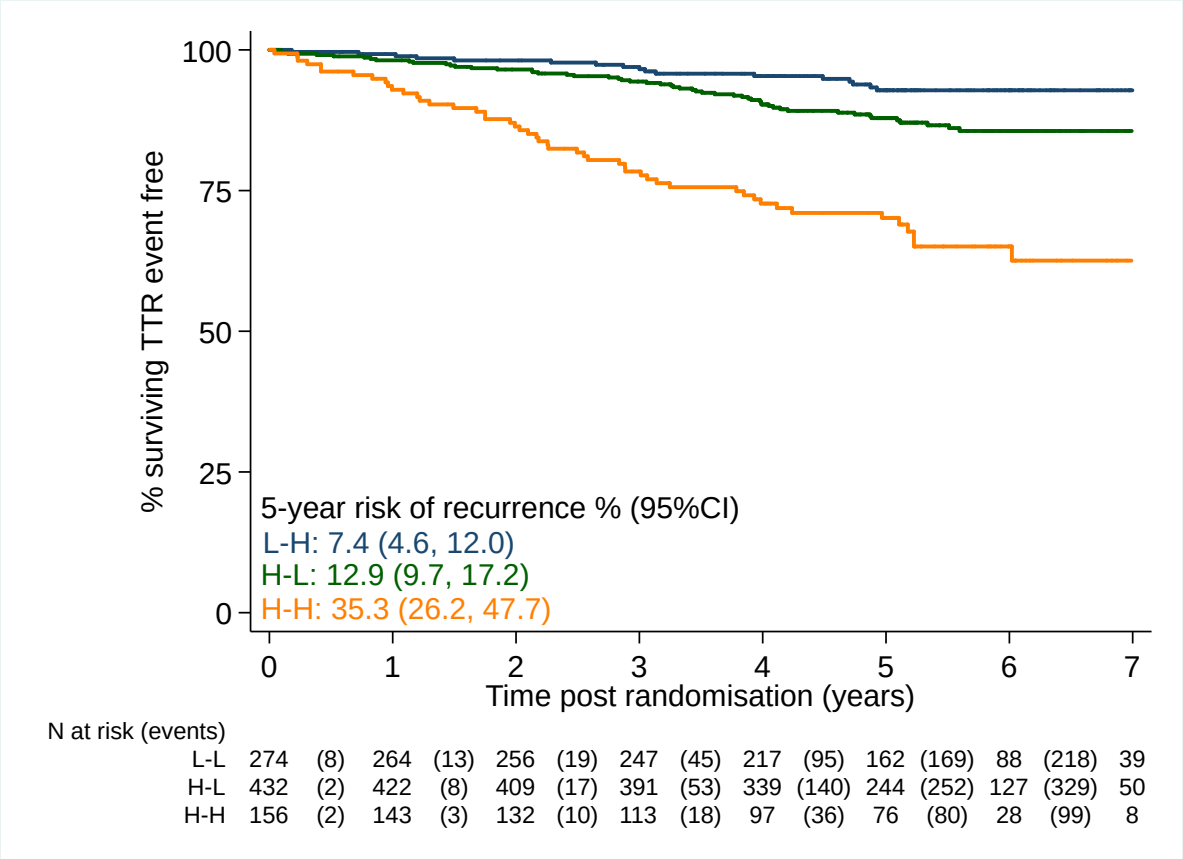
In those with $Ki67_B$≥10% (n=538) unadjusted HR=3·00, 95%CI: 1·98, 4·55; p<0·0001, adjusted HR=2·17, 95%CI: 1·32, 3·59; p=0·0024

Figure A4a: Kaplan Meier survival curve for time to recurrence by $Ki67_B$ and $Ki67_{2W}$ for patients with HR+ and HER2- breast cancer allocated perioperative AI who are <70 years old



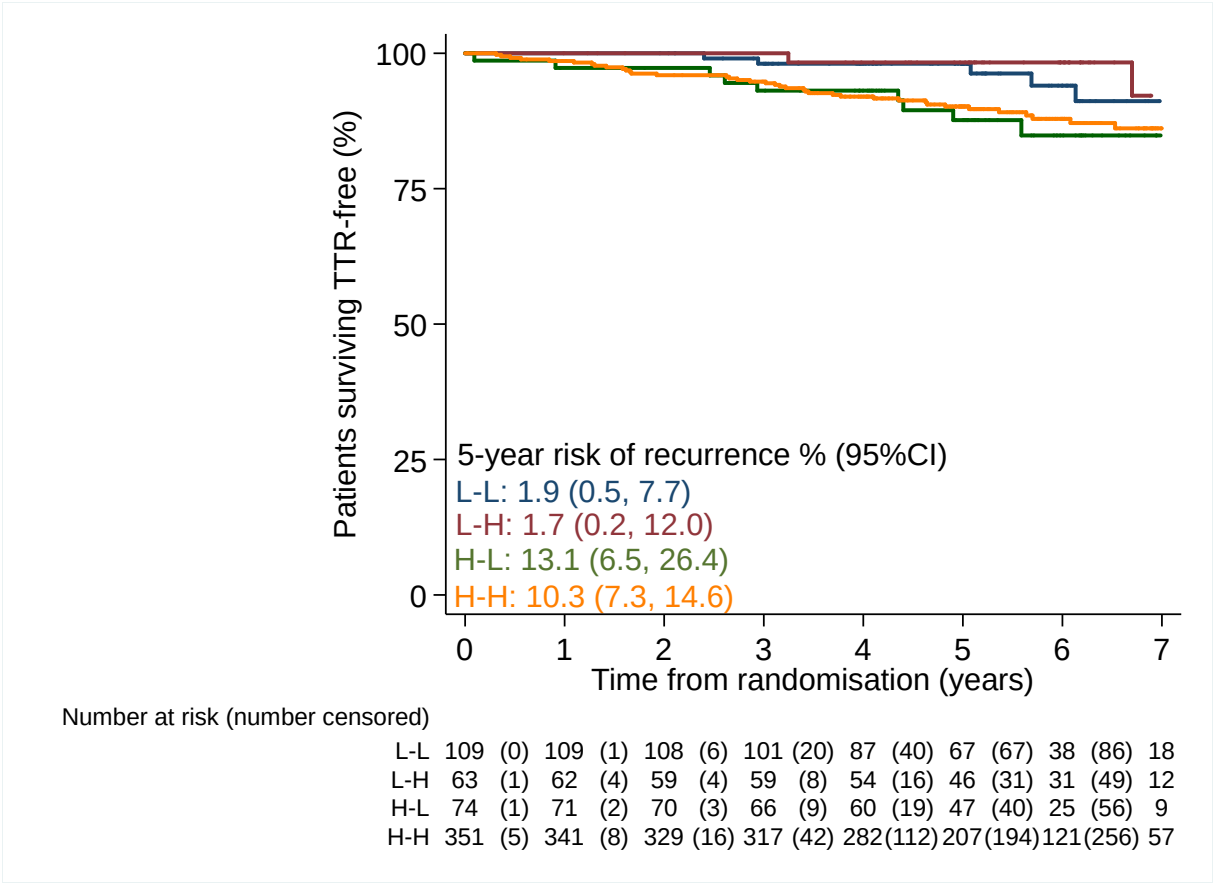
Patients in the L-H group are omitted from the figure n=19 patients

Figure A4b: Kaplan Meier survival curve for time to recurrence by $Ki67_B$ and $Ki67_{2W}$ for patients with HR+ and HER2- breast cancer allocated perioperative AI who are ≥70 years old



Patients in the L-H group are omitted from the figure n=9 patients

Figure A5: Kaplan Meier survival curve for time to recurrence by $Ki67_B$ and $Ki67_{2W}$ for patients with HR+ and HER2- breast cancer allocated control



Unadjusted HR=0·90, 95%CI: 0·44, 1·87; p=0·78, adjusted HR=1·30, 95%CI: 0·52, 3·26; p=0·58

Exploratory analysis

Exploratory analysis of cut-points conducted in the HER2-negative subgroup showed that a cut-point of around 15-20% would be good at discriminating for Ki67B and one around 6-8% would be good for $Ki67_{2W}$.

Dichotomising at Ki67B≥20% and Ki672W≥8% defined 763 (34%) of 2235 patients as H at baseline and 369 (17%) of 2235 and 394 (18%) of 2235 as H-H and H-L, respectively. This compared with 1503 (67%) of 2235 as baseline H and 406 (18%) of 2235 and 1097 (49%) of 2235 as H-H and H-L using our original 10% cut-point. Patients in the H-H group were significantly more likely to have a recurrence than those in the H-L group (unadjusted HR=2·08 (95%CI: 1·43, 3·02); p=0·0001, adjusted HR=1·98 (95%CI: 1·28, 3·08); p=0·0022).

Figure A6a: Exploratory analysis using $Ki67_B$ (20% cut-point) and $Ki67_{2W}$ (8% cut-point) for patients with HR+ and HER2-negative breast cancer allocated perioperative AI - Harrell's C statistic by Ki67 cut-point used

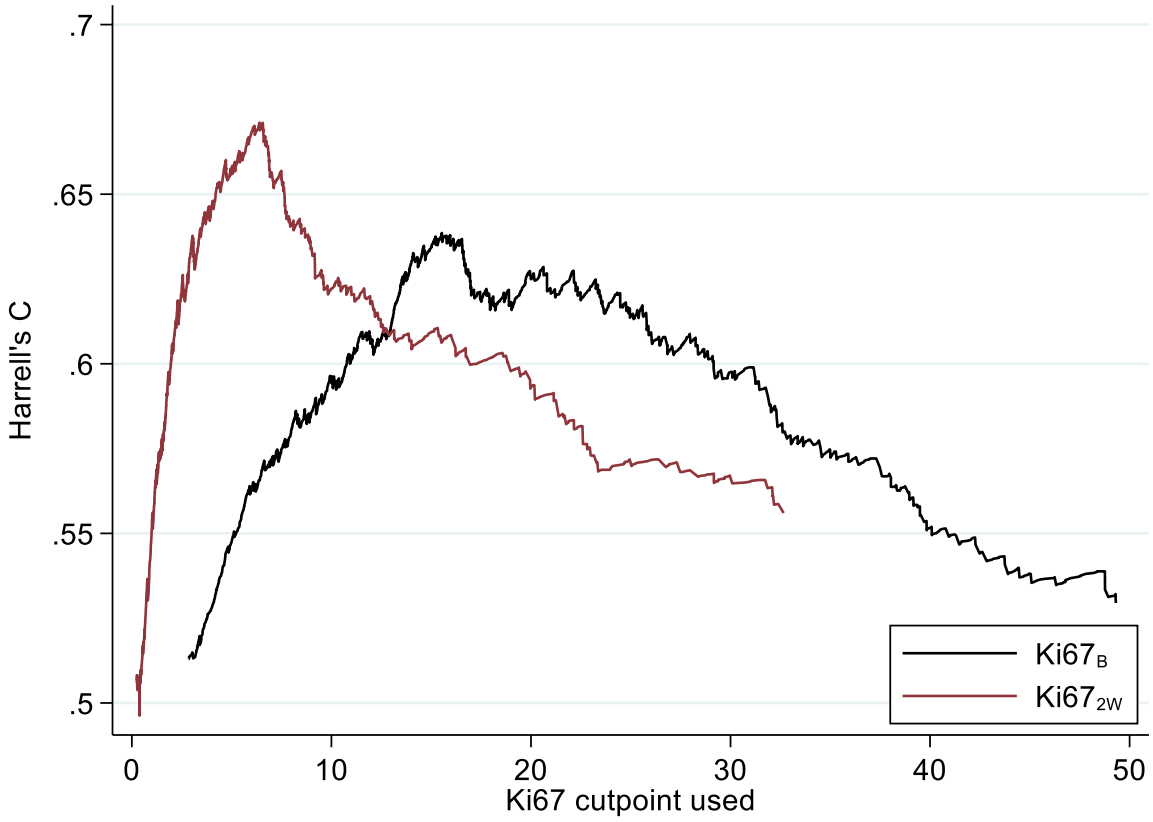
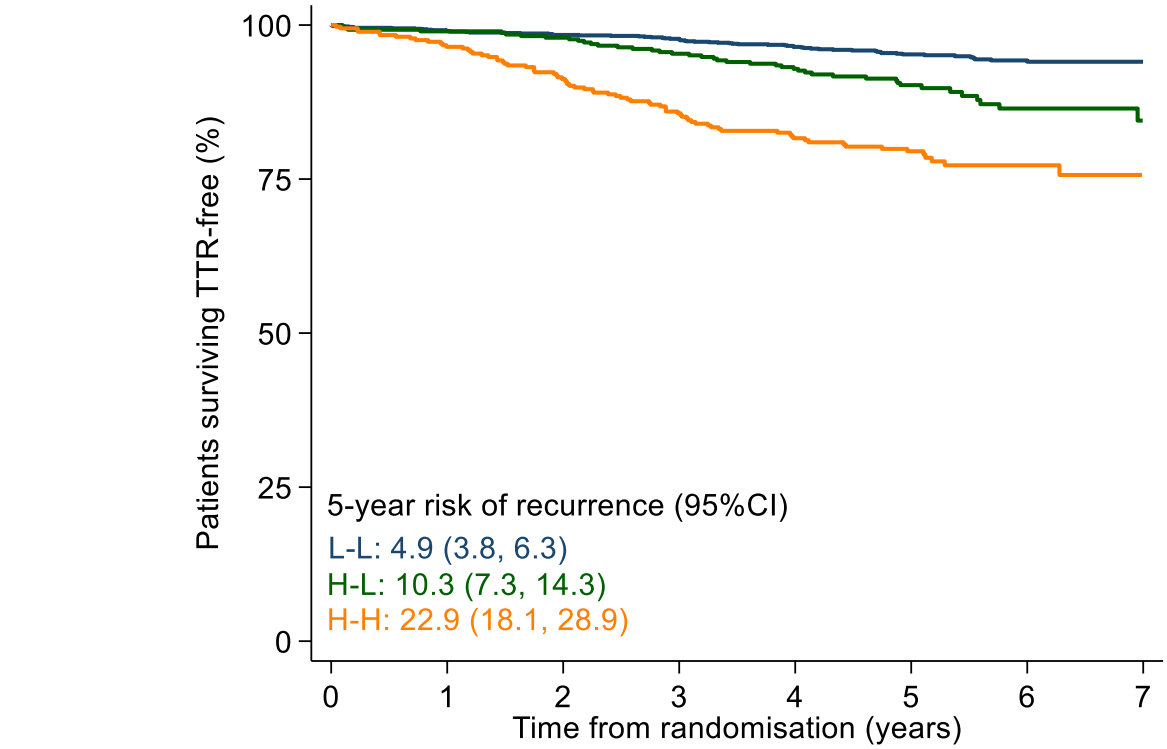


Figure A6b: Exploratory analysis using $Ki67_B$ (20% cut-point) and $Ki67_{2W}$ (8% cut-point) for patients with HR+ and HER2-negative breast cancer allocated perioperative AI - Kaplan Meier survival curve for time to recurrence by $Ki67_B$ and $Ki67_{2W}$

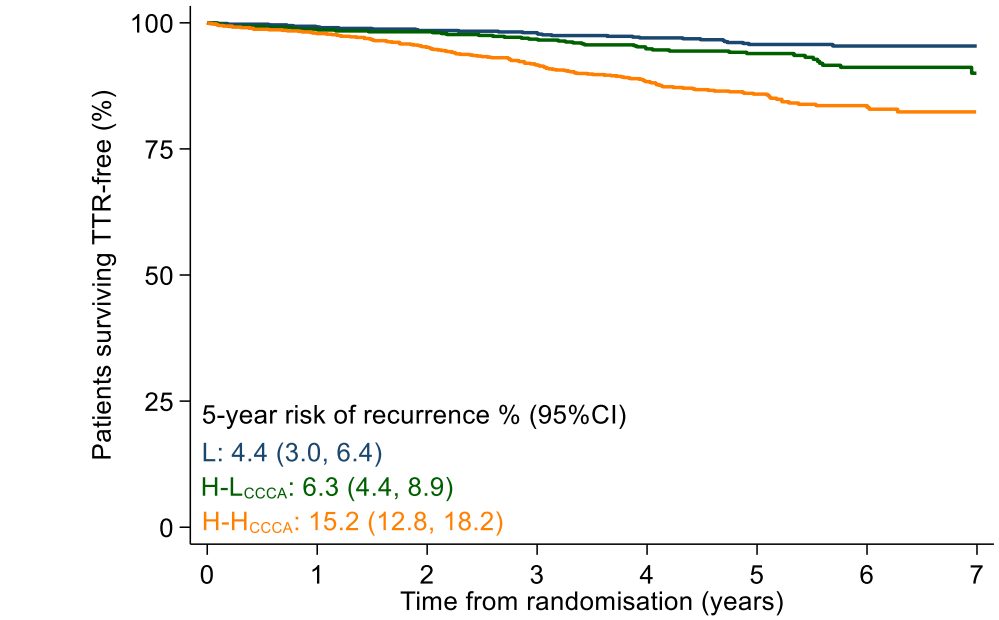


Number at risk (number censored)

L-L	1318	(13)	1293	(30)	1267	(58)	1231	(180)	1093	(457)	804	(812)	443	(1083)	171
H-L	394	(5)	385	(8)	378	(16)	360	(40)	327	(132)	227	(252)	101	(315)	37
H-H	369	(3)	353	(6)	331	(14)	303	(34)	269	(104)	193	(214)	84	(269)	28

Figure A7: Kaplan Meier survival curve for time to recurrence using $Ki67_B$ (10% cut-point) and $Ki67_{2W}$ (CCCA cut-point) for patients with HR+ and HER2- breast cancer allocated perioperative AI

A further unplanned exploratory analysis looking at CCCA after two weeks found that patients in the H-H_{CCCA} group were significantly more like to have a recurrence than those in the H-L_{CCCA} group and that the H-L_{CCCA} group had a risk only modestly higher than those with low baseline Ki67.



N at risk (events)

L	732	(12)	714	(20)	701	(37)	681	(105)	606	(243)	461	(451)	252	(603)	100
"H-L_{CCCA}"	563	(3)	553	(9)	544	(22)	523	(69)	466	(191)	340	(343)	181	(453)	70
"H-H_{CCCA}"	940	(9)	911	(21)	874	(39)	824	(96)	738	(298)	518	(562)	244	(719)	84

CCCA = cell cycle arrest ($Ki67_{2W} \leq 2{\cdot}7\%$)
Unadjusted HR=2·21 (95%CI: 1·56, 3·15); p<0·0001, adjusted HR=1·78 (95%CI: 1·21, 2·62); p=0·0036

Table A1: Protocol compliance by randomised treatment group

	Treatment group	N	Median	IQR	p-value for difference
Time from randomisation to AI start (days)	POAI	2941	1	(0, 5)	N/A
	Control	-	-	-	
Time from start of AI to primary surgery (days)	POAI	2930	14	(13, 14)	N/A
	Control	-	-	-	
Time from primary surgery to post-operative AI completion (days)	POAI	2927	14	(13, 14)	N/A
	Control	-	-	-	
Total time on perioperative AI (days)	POAI	2936	28	(28, 28)	N/A
	Control	-	-	-	
Time from randomisation to surgery (days)	POAI	2953	15	(12, 19)	0.31
	Control	1495	15	(12, 19)	
Number of in patient days for surgery (days)	POAI	2885	1	(1, 3)	0.51
	Control	1475	1	(1, 3)	

Table A2: Adjuvant non-hormone treatment received by randomised treatment group

	Perioperative AI		Control		Total	
	n	***%***	**n**	***%***	**n**	***%***
Adjuvant chemotherapy given?						
Yes	770	*26*	460	*31*	1230	*28*
No	2160	*73*	1025	*69*	3185	*72*
Unknown	27	*1*	8	*<1*	35	*1*
Adjuvant radiotherapy given?						
Yes	2235	*76*	1156	*77*	3391	*76*
Conservative surgery	1902	*64*	992	*66*	2894	*65*
Radiotherapy	1825	*62*	941	*63*	2766	*62*
No radiotherapy	68	*2*	47	*3*	115	*3*
Missing	9	*<1*	4	*<1*	13	*<1*
Mastectomy	1051	*36*	503	*34*	1436	*32*
Radiotherapy	409	*14*	215	*14*	624	*14*
No radiotherapy	630	*21*	286	*19*	916	*21*
Missing	12	*<1*	2	*<1*	14	*<1*
Unknown	27	*<1*	8	*<1*	35	*1*
Other adjuvant treatment given?						
Yes	221	*7*	117	*8*	338	*7*
Anti-HER2 therapy	182	*57*	99	*65*	281	*61*
Other	38	*1*	17	*1*	55	*1*
No	2702	*91*	1364	*91*	4066	*91*
Missing	36	*1*	11	*<1*	47	*1*

Denominators exclude patients where surgery was cancelled and for chemotherapy excludes patients who had a recurrence/second primary prior to the chemotherapy start date (N=7, 3 Perioperative AI, 4 Control) – denominators: Perioperative AI=2957, Control=1493 for other non-hormone treatment excludes patients who had a recurrence/second primary prior to the other non-hormone treatment start date (N=6, 1 Perioperative AI, 5 Control) - denominators: Perioperative AI=2959, Control=1492 and for anti-HER2 therapy also excludes patients who were not HER2+ - denominators: Perioperative AI=317, Control=152

Table A3: Multivariable logistic regression analysis to determine factors affecting chemotherapy use

Models were fitted using a forward stepwise approach.

		OR	95%CI	p-value
Model without post-surgery tumour grade				
Treatment allocation	Control	1·00	-	-
	POAI	1·52	1·26, 1·83	<0·0001
Age group	<70	1·00	-	-
	≥70	0·06	0·04, 0·07	<0·0001
Nodal status	Negative	1·00		
	1-3 nodes positive	5·89	4·81, 7·21	<0·0001
	4+ nodes positive	15·70	11·52, 21·39	<0·0001
Vascular invasion	No	1·00	-	-
	Yes	1·52	1·24, 1·86	<0·0001
Multifocal	Yes	1·00	-	-
	No	0·71	0·55, 0·91	0·0075
Histological type post surgery	Ductal	1·00	-	-
	Lobular	0·67	0·51, 0·88	0·0036
	Other	0·60	0·33, 1·09	0·092
HER2 status	Negative	1·00	-	-
	Positive	11·19	8·51, 14·73	<0·0001
	Not known	0·45	0·13, 1·64	0·23
Pathological tumour size (continuous)		1·37	1·28, 1·47	<0·0001
Model with post-surgery tumour grade				
Treatment allocation	Control	1·00	-	-
	POAI	1·17	0·95, 1·42	0·13
Age group	<70	1·00	-	-
	≥70	0·04	0·03, 0·05	<0·0001
Nodal status	Negative	1·00	-	-
	1-3 nodes positive	7·99	6·40, 9·98	<0·0001
	4+ nodes positive	20·25	14·5, 28·25	<0·0001
Vascular invasion	No	1·00	-	-
	Yes	1·17	0·95, 1·45	0·14
Multifocal	Yes	1·00	-	-
	No	0·65	0·50, 0·85	0·0015
Histological type post-surgery	Ductal	1·00	-	-
	Lobular	0·82	0·61, 1·08	0·16
	Other	0·90	0·47, 1·71	0·75
HER2 status	Negative	1·00	-	-
	Positive	8·76	6·53, 11·74	<0·0001
	Not known	0·46	0·12, 1·71	0·25
Pathological tumour size (continuous)		1·30	1·21, 1·40	<0·0001
Tumour grade	G1	1·00	-	-
	G2	3·67	2·50, 5·40	<0·0001
	G3	18·43	12·09, 28·09	<0·0001

Odds Ratios (OR)>1 favour chemotherapy use

Table A4: Adjuvant hormone therapy plan by randomised treatment group

	Perioperative AI (N=2960)		Control (N=1497)		Total (N=4457)	
	n	***%***	**n**	***%***	**n**	***%***
Adjuvant hormone treatment planned?						
Yes	2909	*98*	1465	*98*	4374	*98*
No	32	*1*	26	*2*	58	*1*
metastatic/second primary/died	*8*	*<1*	*10*	*1*	*18*	*<1*
clinician decision – low risk/no invasive disease	*4*	*<1*	*1*	*<1*	*5*	*<1*
HR-ve on surgery sample	*11*	*<1*	*8*	*1*	*19*	*<1*
patient refusal	*5*	*<1*	*6*	*<1*	*11*	*<1*
other[1]	*4*	*<1*	*1*	*<1*	*4*	*<1*
Missing	19	*1*	6	*<1*	25	*1*
Treatment plan:[2]						
Tamoxifen monotherapy	180	*6*	135	*9*	315	*7*
AI monotherapy	2507	*86*	1186	*81*	3693	*84*
Tamoxifen switching to AI after 2-3yr	144	*5*	107	*7*	251	*6*
AI switching to Tamoxifen	74	*3*	36	*2*	110	*3*
Missing	4	*<1*	1	*<1*	5	*<1*
Did patient complete 5 years adjuvant hormone therapy?[3]						
Yes[4]	1439	*77*	707	*76*	2146	*77*
No	423	*23*	222	*24*	645	*23*
Disease progression or second primary	187	*10*	109	*12*	296	*11*
Death	128	*7*	56	*6*	184	*7*
Side effects incl.menopausal symptoms/adverse event	86	*5*	40	*4*	126	*5*
Never started	2	*<1*	1	*<1*	3	*<1*
Not specified	*20*	*1*	*16*	*2*	*36*	*1*
Treatment received[3]						
Tamoxifen monotherapy	139	*7*	103	*11*	242	*9*
AI monotherapy	1421	*76*	675	*73*	2096	*75*
Tamoxifen switching to AI	107	*6*	52	*6*	159	*6*
AI switching to Tamoxifen	170	*9*	84	*9*	254	*9*
Unknown	25	*1*	15	*2*	40	*1*

The treatment plan details in this table are provided once the adjuvant hormone treatment plan is agreed and after completion of trial treatment or two weeks after surgery if allocated to Control. The actual adjuvant hormone treatment received is then collected at approximately 5 years post-randomisation. The table excludes patients whose surgery was cancelled.

[1]Other reasons given were as follows: Perioperative AI: DCIS only – no invasive cancer, currently having chemotherapy – may start in future, having chemotherapy and radiotherapy, having chemotherapy – no plan to give hormone therapy; Control: no reason documented

[2]Percentages calculated from the number of patients with adjuvant hormone therapy planned (Perioperative AI = 2909, Control = 1465)

[3]Percentages are calculated from the number of patients with actual hormone therapy received data available (Perioperative AI = 1862, Control = 929)

[4]28 patients (Perioperative AI = 15, Control = 13) were continuing beyond 5 years.

Table A5: Maximum CTCAE graded adverse events reported at surgery or the first follow-up visit post-surgery

	Perioperative AI (N=2801)				Control (N=1400)			
	Grade 1-2		Grade 3		Grade 1-2		Grade 3	
	n	%	n	%	n	%	n	%
Hot flushes	1189	*42*	20	*1*	359	*26*	6	*<1*
Sweating	834	*30*	0	*0*	307	*22*	0	*0*
Musculoskeletal pain	1187	*42*	29	*1*	564	*40*	13	*1*

No grade 4 or 5 adverse events were reported.

Table A6: Listing of serious adverse reactions (SARs)

Patient ID	Type of SAR	Age at trial entry	Treatment	Number of days on AI prior to SAR	Severity	Relatedness	Summary	Outcome	Comment
1	Life Threatening	85	Letrozole	29	Life Threatening	Possible	Pulmonary embolism (G4); Atrial fibrillation (G3); Dizziness (G3)	Recovered	CI: Letrozole not associated with increased risk of PE in literature but possible temporal relationship
2	Life Threatening	83	Letrozole	14	Life Threatening	Probable	Atrial fibrillation(G3); Ventricular tachycardia(G3)	Recovered	
3	Life Threatening	57	Letrozole	22	Life Threatening	Possible	Pulmonary embolism (G4)	Recovered with Sequelae	Treated with Warfarin - for 6months + 6weeks post op
4	Life Threatening	64	Letrozole	22	Life Threatening	Probable	Dyspnoea/Shortness of breath (G3)	Recovered	
5	Other	53	Letrozole	8	Severe	Possible	Diarrhoea (G1), Musculoskeletal pain (G3)	Recovered	
6	Other	58	Letrozole	1	Severe	Definite	Eye disorders other – bloodshot (G1); Hypertension (G2); Dizziness (G2); Headache (G2)	Recovered	
7	Other	59	Letrozole	4	Mild	Probable	Hot Flushes (G3); Night sweats (G2); Musculoskeletal pain (G2)	Condition unchanged	Will be on Letrozole for 5 years so condition isn't expected to abate
8	Life Threatening	69	Letrozole	27	Life Threatening	Probable	Thrombus/ thrombosis/embolism (G4)	Recovered with Sequelae	
9	Other	80	Letrozole	4	Moderate	Possible	Musculoskeletal pain (shoulder, hip, leg)(3)	Condition unchanged	
10	Hospitalisation	67	Letrozole	2	Moderate	Possible	Palpitations (G2), Chest tightness (G2)	Recovered	Subsequent diagnosis of E Coli septicaemia
11	Other	69	Letrozole	16	Severe	Possible	Syncope (G3); Hypertension (G2)	Recovered	

Table A7: Absolute risk of recurrence risk at 1, 3 and 5 years by $Ki67_B$ and $Ki67_{2W}$ for patients with HR+ and HER2- breast cancer allocated perioperative AI

$Ki67_B$	$Ki67_{2W}$	TTR events/Number of patients	1 year % (95%CI)	3 year % (95%CI)	5 year % (95%CI)
L	L	29/704	0·7 (0·3, 1·7)	1·9 (1·1, 3·3)	4·3 (2·9, 6·3)
L	H	2/28	3·6 (0·5, 25·4)	3·6 (0·5, 25·4)	7·7 (1·9, 31·.1)
L	**combined**	**31/732**	**0·8 (0·4, 1·9)**	**2·0 (1·2, 3·3)**	**4·4 (3·0, 6·4)**
H	L	95/1097	1·2 (0·7, 2·1)	4·0 (2·9, 5·4)	8·4 (6·8, 10·5)
H	H	83/406	3·5 (2·1, 6·0)	14·3 (10·9, 18·7)	21·5 (17·1, 27·0)
H	**combined**	**178/1503**	**1·8 (1·3, 2·7)**	**6·6 (5·4, 8·1)**	**11·8 (10·1, 13·8)**

L=Ki67 <10%; H=Ki67 ≥10%

Table A8: Median Ki67 values (%) at baseline by combined baseline and 2-week Ki67 group and allocated treatment in patients with HR+ and HER2- breast cancer

		% Ki67 at baseline			
		L-L	**L-H**	**H-L**	**H-H**
POAI	**n**	704	28	1097	406
	Median (IQR)	6·0 (3·9, 8·0)	7·8 (5·5, 8·8)	17·8 (13·5, 25·3)	31·9 (22·2, 45·2)
	p-value for difference	0.033		<0.0001	
Control	**n**	109	63	74	351
	Median (IQR)	6·0 (3·8, 7·8)	6·7 (4·6, 8·7)	14·8 (12·2, 19·2)	23·3 (15·6, 33·1)
	p-value for difference	0.092		<0.0001	

Table A9: Absolute risk of recurrence risk at 1, 3 and 5 years by $Ki67_B$ and $Ki67_{2W}$ for patients with HR+ and HER2- breast cancer allocated control

$Ki67_B$	$Ki67_{2W}$	TTR events/Number of patients	1 year % (95%CI)	3 year % (95%CI)	5 year % (95%CI)
L	L	5/109	0·0 (-, -)	1·9 (0·5, 7·7)	1·9 (0·5, 7·7)
L	H	3/63	0·0 (-, -)	0·0 (-, -)	1·7 (0·2, 12·0)
L	**combined**	**24/382**	**1·3 (0·6, 3·2)**	**2·4 (1·3, 4·6)**	**5·3 (3·3, 8·4)**
H	L	9/74	2·7 (0·7, 10·9)	7·1 (3·0, 17·1)	13·1 (6·5, 26·4)
H	H	39/351	1·4 (0·6, 3·5)	5·4 (3·4, 8·5)	10·3 (7·3, 14·6)
H	**combined**	**86/760**	**2·0 (1·2, 3·3)**	**6·4 (4·8, 8·5)**	**11·1 (8·8, 13·9)**

L=Ki67 <10%; H=Ki67 ≥10%

Table A10: Absolute risk of recurrence risk at 1, 3 and 5 years by $Ki67_B$ and $Ki67_{2W}$ for patients with HR+ and HER2+ breast cancer allocated perioperative AI

$Ki67_B$	$Ki67_{2W}$	TTR events/Number of patients	1 year % (95%CI)	3 year % (95%CI)	5 year % (95%CI)
L	L	3/32	3·1 (0·.4, 22·2)	6·4 (1·6, 25·4)	10·1 (3·2, 31·3)
L	H	2/4	0·0 (-, -)	0·0 (-, -)	25·0 (3·5, 177·5)
L	**combined**	**5/36**	**2·8 (0·4, 19·7)**	**5·6 (1·4, 22·5)**	**12·3 (4·6, 32·9)**
H	L	7/94	1·1 (0·2, 7·6)	2·2 (0·5, 8·6)	7·7 (3·4, 17·5)
H	H	21/143	3·6 (1·5, 8·6)	10·5 (6·2, 17·7)	15·7 (10·1, 24·4)
H	**combined**	**28/237**	**2·6 (1·2, 5·7)**	**7·1 (4·3, 11·5)**	**12·4 (8·4, 18·3)**

L=Ki67 <10%; H=Ki67 ≥10%

Table A11: Sensitivity analysis of absolute risk of recurrence risk at 1, 3 and 5 years by $Ki67_B$ for all patients with HR+ and HER2- breast cancer (perioperative AI and control groups combined)

$Ki67_B$	TTR events/ No. of patients	1 year % (95%CI)	3 year % (95%CI)	5 year % (95%CI)
L	56/1142	1·0 (0·5, 1·8)	2·1 (1·4, 3·1)	4·7 (3·5, 6·3)
H	268/2310	1·8 (1·4, 2·5)	6·4 (5·4, 7·6)	11·5 (10·1, 13·1)

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Royal Liverpool & Broadgreen University Hospitals NHST	Mr Christopher Holcombe	138
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Trial of Perioperative Endocrine Therapy - Individualising Care

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Co-Sponsors:	The Royal Marsden NHS Foundation Trust
	The Institute of Cancer Research
Approval:	Clinical Trials Advisory & Awards Committee (CTAAC)
	Translational Research in Clinical Trials (TRICC)
Funders:	Cancer Research UK
Coordinating Clinical Trials Unit:	The Institute of Cancer Research Clinical Trials and Statistics Unit (ICR-CTSU)
Endorsements:	Association of Breast Surgery at BASO

Version 6 – 8 July 2015

This trial is part of the National Institute for Health Research (NIHR) portfolio as a high priority trial.

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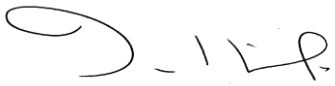
The Trial Management Group (TMG) is constituted from members of the Protocol Development Group and selected principal investigators from participating centres. A copy of the current membership of the TMG can be obtained from the POETIC Trial manager within ICR-CTSU.

Protocol Authorised by:

Name and Role	Signature:	Date:
Professor Ian Smith		08 July 2015

This protocol describes the POETIC trial and provides information about procedures for entering patients. The protocol should not be used as a guide for the treatment of other patients; every care was taken in its preparation, but corrections or amendments may be necessary. These will be circulated to investigators in the trial, but centres entering patients for the first time are advised to contact The Institute of Cancer Research - Clinical Trials & Statistics Unit (ICR-CTSU) to confirm they have the most recent version. Protocol amendments will be circulated to participating centres as they occur.

Queries relating to this trial should be referred, in the first instance, to the POETIC Trial Manager within ICR-CTSU.

This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031). It will be conducted in compliance with the protocol, the Data Protection Act (Z6364106) and other regulatory requirements as appropriate.

POETIC Trial TABLE OF CONTENTS

1. Trial Summary

TITLE:	Trial of Perioperative Endocrine Therapy – Individualising Care (POETIC)
OBJECTIVES:	To determine whether perioperative endocrine therapy with an aromatase inhibitor (AI) followed by standard adjuvant therapy improves outcome compared with standard adjuvant therapy alone in postmenopausal women with hormone receptor positive breast cancer. To determine whether the proliferation marker Ki67 as measured by immunohistochemistry (IHC) in the excised cancer around 2 weeks after starting AI therapy will predict for time to recurrence (TTR) in the individual patient more effectively than the pre-treatment Ki67 value. To determine whether molecular profiling 2 weeks after starting endocrine therapy predicts for long-term outcome in postmenopausal women with hormone receptor positive breast cancer better than at diagnosis.
Exploratory Objectives:	To deconstruct the underlying mechanisms that might explain the development of metastatic disease by correlating the genomic data of primary and metastatic tissue a) with clinical data of patients and b) against Ki67 and other molecular features measured at baseline and 2-week post-treatment. To determine whether the amount of ctDNA in the plasma collected could act as a measure of residual disease after surgery and predictor of relapse.
TRIAL DESIGN:	Phase III, multi-centre, randomised trial. Patients will be allocated in a 2:1 ratio to PERIOPERATIVE THERAPY with an AI for 4 weeks (two weeks before and two weeks after surgery) or NO PERIOPERATIVE THERAPY.
PATIENT TYPE/NUMBER:	Postmenopausal women with ER/PgR positive primary breast cancer. The trial aims to recruit approximately 4350 patients plus the number required to complete POETIC sub-studies.
TRIAL TREATMENT:	Group I: PERIOPERATIVE THERAPY with an AI Choice of AI is according to centre policy and may be either anastrozole (1mg/day) or letrozole (2.5mg/day) Group II: NO PERIOPERATIVE THERAPY
TREATMENT DURATION:	4 weeks (two weeks before and two weeks after surgery)
ENDPOINTS:	**Primary endpoints:** • Time to recurrence (clinical endpoint) **Secondary endpoints:**

- Relapse free survival
- Time to local recurrence
- Time to distant recurrence
- Overall survival
- Breast cancer free survival
- Proliferation rate (Ki67) at baseline core biopsy and at surgical excision (biological endpoint)Gene expression profile at core biopsy, and at surgical excision

Exploratory endpoints

- Gene expression profile at metastatic excision;
- Mutation profiles at core biopsy, surgical and metastatic excision and
- Somatic DNA alterations of ctDNA that might be defined as coming from the primary tumour

2. Introduction

Experimental evidence suggests that endocrine therapy shortly before and immediately after breast cancer surgery might improve outcome (1). This important hypothesis has never been tested clinically. Adjuvant endocrine therapy and adjuvant chemotherapy each result in significant survival benefits in women with early breast cancer (2), but techniques to predict benefit for the individual patient are very poorly developed, and treatment is planned on the basis of probability of the potential to achieve a worthwhile benefit, using standard prognostic and predictive parameters (size, grade, nodal involvement, hormone receptor status, HER2 status and age). A means of predicting outcome of endocrine therapy with molecular techniques in an individual patient would allow much more rational decisions on the need for additional therapy than is currently possible. This would minimise unnecessary toxicity, save on resources, and hopefully improve outcome.

The POETIC trial aims to test these hypotheses in a randomised clinical trial in postmenopausal patients with hormone receptor-positive breast cancer.

3. Background and Rationale

The rationale for systemic perioperative therapy is supported by the fact that access of tumour cells to the circulation is an essential step towards metastasis. A study of 18 patients detected circulating tumour cells in one patient before, in six during, and none after primary breast cancer surgery (3). The clinical significance of this is uncertain, but experimental systems indicate that non-curative reduction of tumour cell burden results in a more rapid doubling time of residual tumour (4). This increase in proliferation rate can be blocked by preoperative tamoxifen (1). More recent clinical work has indicated that two weeks preoperative therapy with an aromatase inhibitor (AI) or tamoxifen markedly reduces proliferation as measured by Ki67 in human breast cancer (5). These observations suggest that short duration perioperative

endocrine therapy might improve long term outcome with no additional toxicity or resource implications. No major clinical trial has so far tested this hypothesis.

The relationship between baseline and two week Ki67 levels and long term outcome was recently analysed in the IMPACT trial comparing neoadjuvant anastrozole with tamoxifen with the combination, and using Ki67 as a primary endpoint (6). It was found that in 158 patients with a median follow-up of 37 months (maximum 88 months) both baseline and 2 week Ki67 levels were significantly related to relapse free survival but the relationship was stronger for 2 week Ki67 (p=0.008). Multivariate analysis showed a highly significant relationship between 2 week but not baseline Ki67 and relapse free survival (7). If these results can be confirmed, then it provides the opportunity for Ki67 measurement in the excised breast cancer in an individual patient treated with 2 weeks preoperative endocrine therapy to contribute to the assessment of the likely outcome in that patient. This would aid greatly in deciding whether additional adjuvant chemotherapy was required for that patient.

Advances in molecular techniques applied to human tissues have allowed the creation of numerous molecular signatures which appear to predict clinical outcome better than standard clinical-pathological markers (8). All of these profiles for breast cancer have been developed on untreated tumours yet they generally attempt to predict the outcome of patients who are then treated with a medical intervention, most frequently endocrine therapy. Two large trials to test the application of different molecular profiles are about to be initiated: MINDACT (9) and TAILORx (10). We have recently demonstrated however that the molecular profile of tumours treated with AIs is radically altered with the expression of over 2,000 (median) genes being significantly changed with 2 weeks treatment (11). These changes vary markedly between patients and it is rational that this may reflect the benefit derived from therapy. Thus it is possible that the recent data indicating that Ki67 measured after 2 weeks preoperative AI treatment may aid in predicting outcome on adjuvant endocrine therapy more accurately than Ki67 measured prior to AI treatment may be extendable to such molecular profiling. At the Royal Marsden Hospital, we have available c.100 pairs of tumour biopsies from ER+ patients taken from patients receiving anastrozole in our recently reported anastrozole±gefitinib neoadjuvant trial to extend our molecular findings to assist in the creation of an on-treatment molecular predictive profile (12). The molecular profile will be developed whilst recruitment to POETIC is ongoing. Samples will be taken from patients in POETIC to allow subsequent extension and validation of the profile.

In the absence of diagnostic tools that characterise whether the disease has been completely eradicated by surgery, the promising approach of circulating tumour DNA measurement as a personalised marker of minimal residual disease will be assessed

Acknowledging the importance of unravelling the mechanisms underlying breast cancer metastasis, gene molecular profile at metastasis against pre-treatment and post-treatment data will be studied.

The POETIC study assesses the benefit of peri-operative non-steroidal AI therapy using anastrozole or letrozole. Exemestane is excluded not for efficacy issues, rather because its steroidal structure may confound biological studies (13, 14).

3.1 Consultation on trial procedures and conduct

The perioperative setting of this study, together with the incorporation of primary biological endpoints, represents a new direction for UK breast cancer research, and will require changes in standard practice within many centres. We have therefore consulted widely with both clinical colleagues and consumers to minimise changes to routine clinical practice, and ensure that the procedures and their timing are acceptable and practical.

Consultation with clinical colleagues

A focus group of interested clinicians and nurses met in October 2007 to identify any potential obstacles to the running of the study. This group highlighted the requirement for tissue taken from patients prior to their entry into POETIC to be stored within an HTA licensed laboratory. As a result of this meeting Pathway B (see Section 8) was introduced to accommodate patients diagnosed prior to having the opportunity to consider the trial. This group also identified ways of improving the flowcharts showing the patient pathways [Appendix 2].

Further to discussions in June 2008 at meetings of the UK Breast Intergroup and the NCRI's Breast Clinical Studies Group, a more pragmatic definition of essential tissue for this study has been developed, in order that only minimal changes to local standard practices and procedures are required to facilitate participation.

Consultation with consumers

Issues pertinent to consumers were presented to the NCRI consumer liaison group on 25 January 2007, and the patient information sheet reflects comments made at that meeting. Consumers were asked to consider whether research tissue should be taken from patients at the same time as diagnostic tissue, or taken after a diagnosis of ER positive breast cancer is confirmed. They were made aware that research tissue taken at diagnosis may need to be taken from patients who subsequently turn out to be ineligible for trial entry, and that taking tissue at that time allowed little time for patients to consider whether they wanted to donate tissue. The consumers preferred extra tissue for research at diagnosis to be taken at the same time as the routine core biopsy, and not after diagnosis is confirmed. Consumers also preferred any tissue taken to be used for research wherever possible.

Views of the North Trent Consumer Research Panel have been sought on the information sheet for donation of tissue at diagnosis.

The POETIC patient information sheets and consent forms, with earlier consumer comments incorporated, were reviewed by the Surrey, West Sussex and Hampshire Cancer Research Network Cancer Partnership Research Group. This group was also asked to consider the appropriate timeframes for patients to consider trial entry. Its view was that the diagnosis should be known before the trial is offered, and length of time available to consider trial entry was of secondary importance.

4. Trial Objectives

1. To determine whether perioperative endocrine therapy with an AI followed by standard adjuvant therapy improves outcome compared with standard adjuvant therapy alone in postmenopausal women with hormone receptor positive breast cancer.

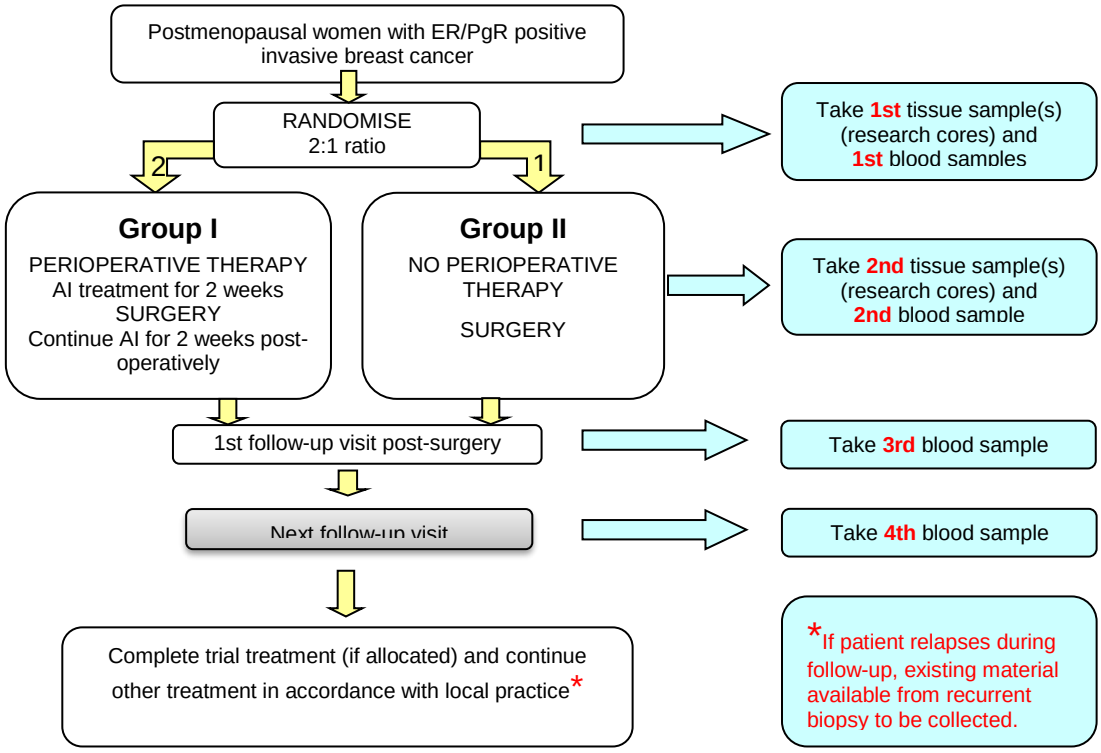
2. To determine whether the proliferation marker Ki67 as measured by immunohistochemistry (IHC) in the excised cancer around 2 weeks after starting AI therapy will predict for time to recurrence (TTR) in the individual patient more effectively than the pre-treatment Ki67 value.
3. To determine whether molecular profiling 2 weeks after starting endocrine therapy predicts for long-term outcome in postmenopausal women with hormone receptor positive breast cancer better than at diagnosis.

4.1 Exploratory Objectives:

1. To deconstruct the underlying mechanisms that might explain the development of metastatic disease by correlating the genomic data of primary and metastatic tissue a) with clinical data of patients and b) against Ki67 and other molecular features measured at baseline and 2-week post-treatment.
2. To determine whether the amount of ctDNA in the plasma collected could act as measure of residual disease after surgery and predictor of relapse.

5. Study Design

For all patients who consent to enter POETIC, surgery should be booked prior to randomisation.



Choice of AI (anastrozole or letrozole) is to be declared by each participating centre at the outset of the trial.

For the purposes of national cancer waiting times, allocation to AI or no AI (following randomisation into POETIC) is classed as first treatment.

In order to maintain the scientific integrity of the trial it is essential that all non trial treatments are not influenced by the patient's allocation in POETIC (e.g. need to ensure that use of adjuvant AIs or chemotherapy does not differ between the two groups). In order to achieve this all non trial treatment should be given according to standard local policy.

6. Endpoints

6.1 Primary endpoints:

- Time to recurrence

6.2 Secondary endpoints:

- Relapse free survival
- Time to local recurrence;
- Time to distant recurrence;
- Overall survival;
- Breast cancer free survival;
- Proliferation rate (Ki67) at baseline core biopsy, and at surgical excision and
- Gene expression profile at core biopsy and at surgical excision.

6.3 Exploratory Endpoints

- Gene expression profile at metastatic excision;
- Mutation profile at core biopsy, surgical and metastatic excision and
- Somatic DNA alterations in the ctDNA that might be defined as coming from the primary tumour.

7. Patient Selection and Eligibility

7.1 Source of patients

Postmenopausal women with ER and/or PgR positive primary breast cancer will be recruited from breast cancer clinics within participating UK centres.

Hormone receptor positive breast cancer is defined as an Allred score of 3 or more, *or* a Histoscore of 2 or more, *or* more than 1% of positive cells for oestrogen receptor or progesterone receptor.

7.2 Number of patients

Approximately 4350 patients will be required plus the number required to complete POETIC sub-studies.

7.3 Inclusion criteria:

1) Post-menopausal women with core biopsy-proven hormone receptor positive invasive breast cancer. Postmenopausal is defined as a woman aged ≥50 years fulfilling any one of the following criteria:
   i) with amenorrhoea >12 months and an intact uterus;
   ii) has undergone a bilateral oophorectomy;
   iii) in women who have undergone a hysterectomy, then FSH levels within the postmenopausal range (utilising ranges from the testing laboratory facility) are required if the patient is aged <55 years; or
   iv) in women who have been on HRT within the last 12 months and therefore not amenorrhoeic, FSH levels within the postmenopausal range (utilising ranges from the testing laboratory facility) are required if the patient is aged <55 years.

2) No evidence of metastatic spread by standard assessment according to local guidelines
3) Standard adjuvant endocrine therapy indicated
4) A palpable tumour of any size , or a tumour with an ultrasound size of at least 1.5cm
5) WHO performance status of 0 or 1
6) Written informed consent to participate in the trial and to donation of tissue (fresh tissue and surplus tissue from diagnostic procedures) and blood samples.

7.4 Exclusion criteria:

1) Locally advanced/inoperable breast cancer
2) Evidence of metastatic disease
3) Previous invasive breast cancer (surgically treated DCIS or LCIS allowed)
4) Current bilateral breast cancer
5) Multiple unilateral tumours with different ER/PgR/HER2 status, grade or type (e.g. ductal vs lobular) i.e. anything that suggests two or more different cancers. Multifocal disease with homogenous ER/PgR/HER2 status, grade and type is allowed if at least one lesion is palpable or at least 1.5cm on ultrasound; the largest lesion should be used for sample collection and CRF completion.
6) Concurrent use (defined as use within 4 weeks prior to diagnostic tissue sample being taken) of HRT or any other oestrogen-containing medication (including vaginal oestrogens)
7) Previous use of oestrogen implants at ANY time
8) Prior endocrine therapy or chemotherapy for breast cancer
9) Any invasive malignancy diagnosed within previous 5 years (other than basal cell carcinoma or cervical carcinoma in situ)
10) Any severe co-incident medical disease, inability to give informed consent or unavailability for follow-up
11) Treatment with an unlicensed or investigational drug within 4 weeks before randomisation
12) Current, continuous, long term systemic steroid usage

8. Procedure for Obtaining Consent for Trial Entry & Tissue Donation

Selected centres will be designated as Biological Centres. Centres in England Wales and Northern Ireland will adhere to guidance from the Human Tissue Authority on taking and storing tissue for patients prior to their entry into POETIC, details of which are available from the POETIC trials office. Biological centres will provide samples in RNA-later® and one paraffin block for all consenting patients, taken prior to study entry and at surgery. Biological centres may enter patients into the study via Pathway A or Pathway B. They may also enter patients as 'non-biological' where individual circumstances make Pathways A and B impractical.

Pathway A: requires tissue to be taken from patients at the same time as the diagnostic core biopsy. Generic consent for research core biopsy samples should be gained prior to diagnostic core biopsy. The patient should be offered the trial as soon as hormone receptor positive breast cancer and eligibility are confirmed.

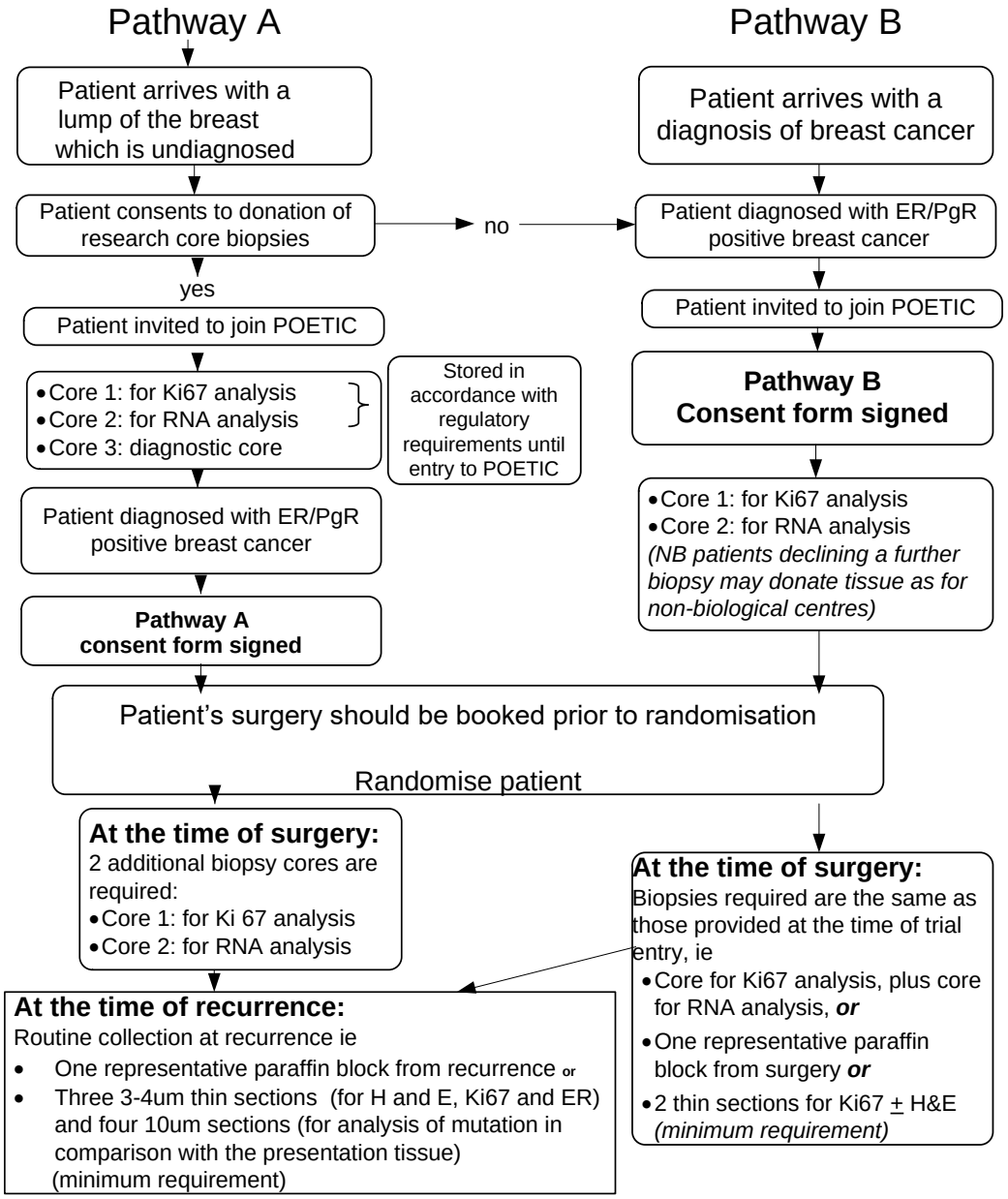
Pathway B: requires patients to be asked to donate additional research core biopsy samples taken after written informed consent and before randomisation.

All other centres (i.e. non-biological centres) are not required to provide samples in RNA-later®. Paraffin embedded tissue must be available from diagnostic tissue already taken.

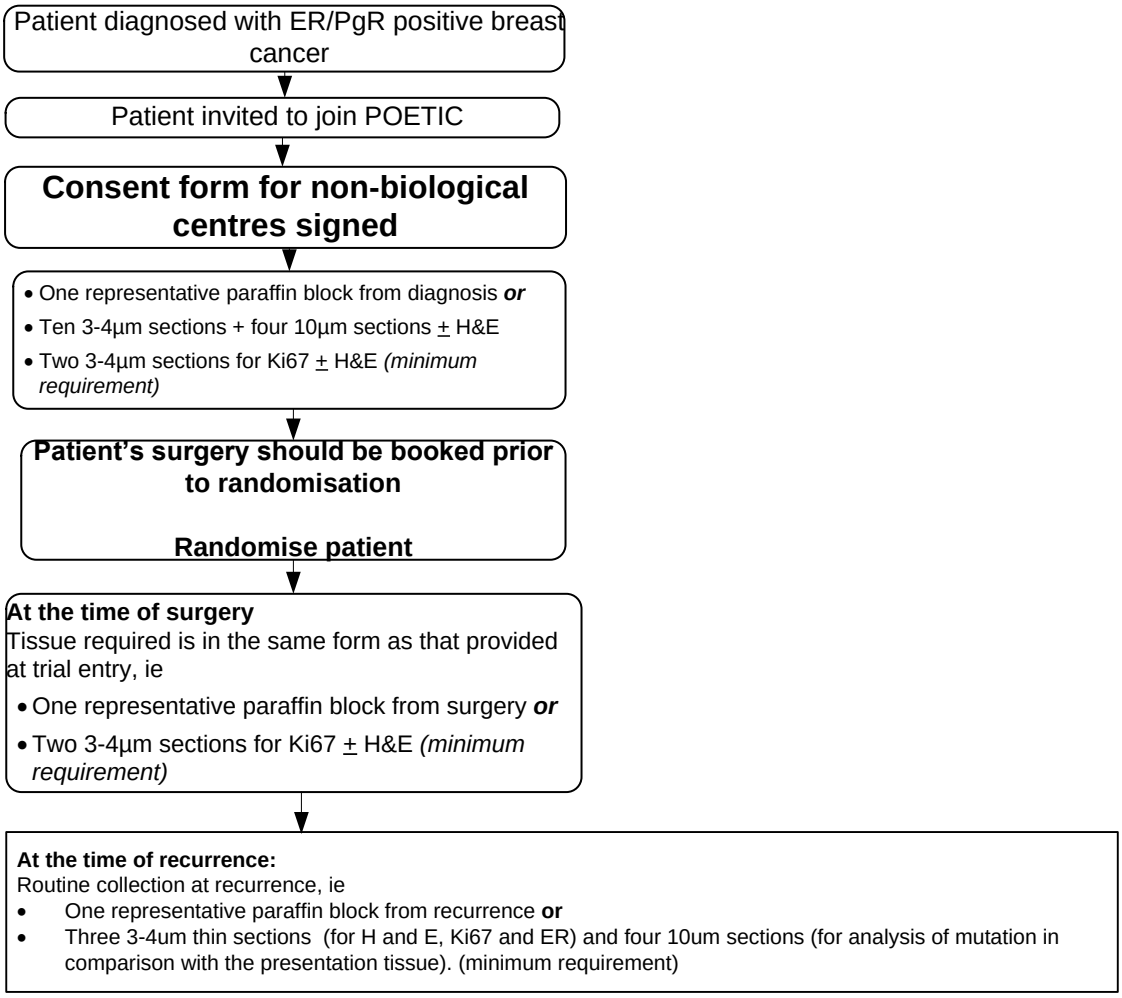
Flowcharts showing the details for both biological and non-biological centres are provided in Appendix 2. Details of samples required for all pathways are provided in Appendix 3

All patients must have their excisional surgery booked prior to randomisation, and it is recommended that patients considered for POETIC are booked for their excisional surgery at the time of first referral.

Biological centres



All other centres (non-biological centres)



9. Biological Specimen Collection

9.1 Specimen Collection

Tissue Collection

The collection of biological samples is an essential part of this study. The minimum requirement is for paraffin embedded tissue to be provided for each patient both prior to trial entry and at surgery. An additional (optional) paraffin embedded tissue sample or sections from routine excision at the time of recurrence, are also requested. Options for the provision of this material are given in the diagrams above and in further detail in Appendix 3.

Centres are encouraged to provide additional material in tubes containing RNA-later® for additional biological studies. Those that provide this material are designated as Biological Centres. As well as paraffin embedded material, Biological Centres will provide one fresh sample in a tube containing RNA-later® (will be provided), taken before randomisation (at diagnosis for Pathway A; at trial entry for Pathway B), for all consenting patients whose tumour size permits multiple biopsies, Biological Centres will also provide a further fresh tissue sample in RNA-later® taken at surgery.

TRICC funding will facilitate the collection of fresh material for the secondary biological analyses on RNA, however subsequent scientific studies will only be undertaken once the scientific procedures have been agreed with the TMG and additional grant funding has been secured.

Blood Sample Collection

The following blood samples are required for all consenting patients:

I. **At randomisation**: two samples;1 x 5ml EDTA, 1 x 8.5ml PAXgene tube
II. **At surgery:** one 5ml sample in EDTA
III. **At first follow-up after surgery**: one 5ml EDTA sample
IV. **Late follow-up visit:** two samples; 1 x 5ml EDTA, 1 x 10ml Streck tube (not required if patient has relapsed)

Blood kits required for the study will be provided, and all centres should participate in the collection of blood samples.

Further details on storage and transfer of blood and tissue samples are given in Appendix 3, and full details of blood and tissue sampling collection and storage are given in trial specific Trial Guidance Notes, which should be followed for all samples taken in relation to the POETIC trial.

9.2 Use of tissue sample collection beyond POETIC

It is envisaged that all tissue taken at diagnosis from patients who do not ultimately enter POETIC is made available for other high quality ethics approved research. To this end, information offered to patients at the time of donating this tissue should make it clear that donated tissue may be made available to researchers.

10. Randomisation Procedure

Patients agreeing to enter the trial will have surgery booked prior to randomisation and scheduled for around two weeks ahead.

HRT users must stop HRT a minimum of four weeks before the baseline diagnostic biopsy is taken (HRT may affect tumour proliferation and hence the Ki67).

For both pathways A and B: patients should ideally be randomised around 14 days before their scheduled surgery date. Patients will be randomised into 2 groups in a 2:1 ratio as follows:

- PERIOPERATIVE THERAPY with AI and excisional surgery 14 days later (or a minimum of 10 days later);

or

- NO PERIOPERATIVE THERAPY with surgery at around 14 days later (or a minimum of 10 days later)

An eligibility checklist and randomisation checklist should be completed prior to randomisation. To randomise a patient, telephone ICR-CTSU on the dedicated randomisation line (see below). The person randomising the patient will be asked to confirm that an eligibility checklist

has been completed and to verify that the patient has signed the POETIC consent form (a sub-set of these will be the subject of a later audit). The person will also be asked for all the information on the randomisation checklist. A trial number and treatment allocation will be given over the telephone and later confirmed in writing.

The eligibility and randomisation checklists should be completed prior to randomisation.

To randomise a patient please telephone:

+44 (0)20 8643 7150 (Monday – Friday 09:00 – 17:00)

11. Trial Treatment

Allocation of treatment

Perioperative endocrine therapy, if allocated, should be started as soon as possible after randomisation.

Irrespective of treatment allocation, patients should not take any concomitant oestrogen-containing medication.

Choice of Investigational Medicinal Product (IMP)

PERIOPERATIVE THERAPY will be either

anastrozole 1mg/day

or letrozole 2.5 mg/day

Anastrozole is used outside its current licensed indication and letrozole is used within its licensed indication. Both are oral preparations formulated as tablets.

Participating centres should declare prior to enrolling patients whether they propose to use anastrozole or letrozole. Centres may, if they wish, change their practice to the alternative AI during the course of the trial, but should do so for all future patients. Before a change is made, the date of changing to an alternative AI should be agreed with ICR-CTSU.

Duration of treatment

Treatment should commence immediately after randomisation allowing duration of treatment before surgery to be as close as possible to 14 days. Ideally, patients should commence trial treatment exactly 14 days before surgery. Where necessary, a minimum of 10 days treatment before surgery is allowed in order to avoid delays to surgery. If surgery is delayed, the pre-treatment duration should be extended, and treatment continued at this daily dose without interruption until 14 days after the date of surgery, including the day of surgery.

12. Drug supplies & labelling

Normal commercial supplies should be used.

Study drug should normally be dispensed by the hospital pharmacy. For anastrozole, patient identifiers and local investigator details should be added to the label in accordance with Annex 13 (*Volume 4, Good manufacturing practices, Annex 13, Manufacture of investigational medicinal products)* by the local pharmacy. Letrozole is being used within its licensed indication therefore it can be labelled in accordance with the requirements for a dispensed medicine. All IMPs should also be labelled 'for clinical trial use only'. Labelling requirements do not apply for study drug dispensed by a community pharmacy, however full drug accountability is required as detailed in the Trial Guidance Notes.

13. Non Trial Treatment

It is important to ensure that use of non-trial therapy, including consideration of participation in an adjuvant treatment trial, is not influenced by the patients' treatment allocation within POETIC. If such a practice occurred, with differential use of adjuvant therapy between the groups, this would undermine the scientific integrity of the trial and affect its ability to reach its stated objectives. In order to avoid this, all non-trial therapy should be given according to standard local practice guidelines. All Non Trial Treatment, as described below, must be recorded in the Case Report Forms (CRF). CRF completion guidelines are contained in the Trial Guidance Notes.

13.1 Surgical Treatment

Patients may undergo either breast conserving surgery or mastectomy in accordance with local protocols and patient choice. Patients entered into this study must have subsequent confirmation of axillary node status. The axilla should be staged by axillary sampling, sentinel node biopsy (SNB) or axillary clearance. If sampling or SNB identifies axillary node involvement, axillary nodes should be resected or axillary radiotherapy undertaken in accordance with local protocols. In patients treated by breast conservation, clear margins should be achieved. Further re-excision to achieve a margin of clearance should be in accordance with local protocols. Where there is a close or involved deep margin following mastectomy, or axillary lymph node involvement, chest wall radiotherapy may be administered in accordance with local protocols (see below).

Primary breast reconstruction and other oncoplastic procedures to improve cosmetic outcome are acceptable in the POETIC trial.

13.2 Radiotherapy

Radiotherapy should be given, if required, after chemotherapy or surgery in keeping with local practice.

13.3 Adjuvant Endocrine therapy

All patients will be treated in accordance with local policy based on the national clinical guidelines that prevail at the time. It is expected that these patients will receive adjuvant endocrine therapy with either tamoxifen or an aromatase inhibitor, or each sequentially, for a

minimum of 5 years. It is recognised that choice and duration of endocrine therapy is a changing field and national policies may change during the course of the trial. Centres must declare their current policy prior to participation, and advise ICR-CTSU of any changes to that policy as they occur. For patients randomised to perioperative endocrine therapy continued treatment with the same AI in the adjuvant setting is not a study requirement.

13.4 Adjuvant Chemotherapy

Chemotherapy and supporting medications (including GCSF) may be offered in accordance with local policy. It is recommended that MDTs give due consideration to other factors, including grade at diagnostic core, when considering adjuvant chemotherapy for patients who have received perioperative AI treatment. This is because grade at surgery following two weeks of perioperative AI treatment may not be as reliable an indicator as it might otherwise have been.

13.5 Adjuvant Herceptin and Bisphosphonates

Adjuvant Herceptin should be given in accordance with national clinical guidelines.

At the time of preparing the POETIC protocol, there are no standard guidelines for adjuvant bisphosphonates; however evidence may emerge during the course of the trial to justify their use. Should such evidence emerge, adjuvant bisphosphonates should be given, if required, in accordance with agreed national guidelines**.**

14. Trial Evaluations

14.1 Staging investigations

Required staging investigations are according to local practice and thus are in keeping with standard UK practice in breast cancer management. No additional staging investigations are required.

14.2 Follow–up Investigations

Patients should be followed-up as per local practice for patients with early breast cancer who have been entered into a clinical trial. Follow-up data will be collected on annual follow-up CRFs, local/distant relapse CRFs, secondary cancer CRFs and death CRFs. Imaging and biochemical investigations will be carried out as clinically indicated where recurrence or other significant clinical problems are suspected.

To enable the 4th blood sample (late follow up) to be collected patients should be invited to attend clinic to consent and collect this sample.

15. Pharmacovigilance

15.1 Definitions

15.1.1 Serious Adverse Events (SAEs)

An SAE is any untoward medical occurrence that occurs after the commencement of randomised treatment and within 30 days of the last administration of the trial drug and:

- Results in death;

- Is life-threatening: *refers to an event in which the patient was at risk of death at the time of the event. It also refers to an event that would result in death with the continued use of the product; it does not refer to an event which hypothetically might have caused death if it were more severe;*
- Requires inpatient hospitalisation or prolongation of existing hospitalisation: *admission to hospital overnight or prolongation of a stay in hospital was necessary as a result of the AE. Outpatient treatment in an emergency room is not itself an SAE, although the reasons for it may be. Hospital admissions/surgical procedures planned for a pre-existing condition before a patient is randomised to the study are not considered SAEs, unless the illness/disease deteriorates in an unexpected way during the study*;
- Results in persistent or significant disability/incapacity: *results in a significant or persistent change, impairment, damage or disruption in the patient's body function/structure, physical activities or quality of life;*
- Is a congenital anomaly/birth defect; or
- Any untoward medical occurrence requiring medical intervention to prevent permanent impairment or damage.

15.1.2 Serious Adverse Reactions (SAR)

A SAR is an SAE that has a definite, probable or possible causal relationship to the trial drug.

15.1.3 Suspected Unexpected Serious Adverse Reactions (SUSARs)

Any adverse reactions that have a suspected relationship to an IMP that are both serious and unexpected, as judged by the CI.

15.2 Causality (relatedness)

The assignment of causality for serious adverse events should be made by the investigator responsible for the care of the patient using the definitions in Table 1. If any doubt about the causality the investigator should inform ICR-CTSU who will notify the Chief Investigator. Pharmaceutical companies and/or other clinicians may be asked to advise.

Table 1 – Definitions for causality

Relationship	Description
Unrelated	There is no evidence of any causal relationship with the trial drug
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment)
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have

	contributed to the event (e.g. the patient's clinical condition, other concomitant treatments)
Probable	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely
Definitely	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship

15.3 Reporting of Serious Adverse Events/Reactions to ICR-CTSU

SAEs in this study that are fatal or life threatening or result in persistent or significant disability/incapacity should be reported using a Serious Adverse Event (SAE) form. Other SAEs that are definitely, probably, or possibly related to the randomised treatment (including prolongation of existing hospitalisation) should be reported using a Serious Adverse Reaction (SAR) form. SAEs that are hospitalisation or prolonged hospitalisation and unrelated or unlikely to be related to the study drug do not require reporting.

Medical and scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or result in persistent or significant disability/incapacity.

Data on adverse events that are not serious in accordance with the above definition will not be collected.

15.4 Recording and Reporting of Serious Adverse Events

Serious adverse events, whether on an SAE or a SAR form, require immediate reporting by fax to ICR-CTSU within 24 hours of the Principal Investigator or designated representative becoming aware of the event.

Please fax SAE and SAR forms for the attention of POETIC Trial Manager to the ICR-CTSU Safety Desk
Fax: +44 (0)20 8722 4368 (Monday – Friday 09:00 – 17:00)

Forms must be completed, signed and dated by the Principal Investigator or designated representative.

15.5 Review of Serious Adverse Event

Events reported using an SAE form will be reviewed immediately by the Chief Investigator (or designated representative) for causality and expectedness.

Centres should respond as soon as possible to requests from the CI or designated representative (via ICR-CTSU) for further information that may be required for final assessment.

15.6 Expedited Reporting of SUSARs

If an SAE is defined as a SUSAR and is fatal or life threatening, ICR-CTSU will report this to the MHRA, the Main REC, and to the Co-Sponsors within 7 days from the date of definition.

If an SAE is defined as a SUSAR and is not fatal or life threatening, ICR-CTSU will report this to the MHRA and Main REC and to the Co-Sponsors within 15 days.

The Principal Investigator at all actively recruiting centres will be informed of any SUSARs occurring within the trial.

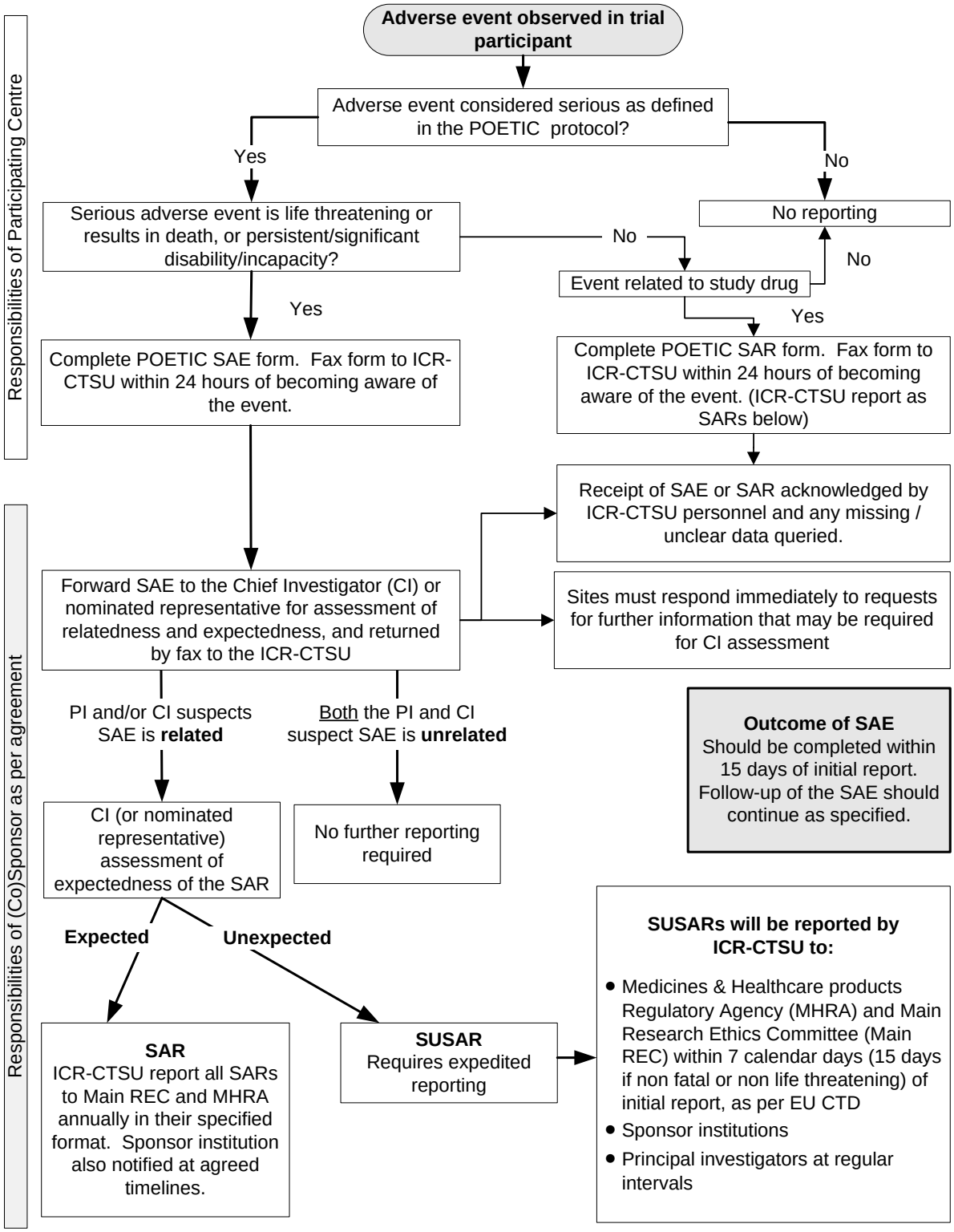
15.7 Follow-up of Serious Adverse Events

The patient must be followed-up until clinical recovery is complete and laboratory results have returned to normal, or until disease has stabilised. Information on final diagnosis and outcome of SAE which may not be available at the time the SAE was initially reported should be completed on the relevant part of the original SAE form within 15 days of resolution of the event and faxed to ICR-CTSU. This applies to all reported SAEs, whether reported on an SAE form or a SAR form.

15.8 Annual Reporting of Serious Adverse Reactions

An annual report will be provided to the MHRA and the Main REC at the end of the reporting year. This will be defined as the anniversary of the date when the Clinical Trials Authorisation (CTA) was obtained. This will include all related events reported on SAE and SAR forms, and a report from the Independent Data Monitoring Committee (IDMC).

Flow diagram for SAE reporting, and action following report



16. Statistical Considerations

16.1 Trial Hypothesis

POETIC is a randomised phase III clinical trial addressing the hypotheses:

- that 4 weeks perioperative treatment with an aromatase inhibitor will improve the time to recurrence in women with ER &/or PgR positive breast cancer compared with standard adjuvant treatment; and
- that the proliferation marker Ki67 following 2 weeks of aromatase inhibitor therapy can predict for long-term outcome in postmenopausal women with hormone receptor positive breast cancer better than at diagnosis.

16.2 Stratification

Randomisation will be stratified by:

- Participating Centre

Stratification by nodal status or grade is not possible as this will not be known at the time of randomisation. With approximately 4350 patients recruited, imbalances in prognostic factors between the treatment groups are unlikely.

Prognostic information on number of clinically involved nodes, Ki67, ER status, PgR status, HER2 status, clinical tumour size and grade will be recorded and may be used to adjust analyses.

16.3 Randomisation

Patients will be allocated in a 2:1 ratio (Perioperative therapy : No perioperative therapy) and randomisation will be conducted according to variable sized permuted blocks. A 2:1 allocation maximises information about perioperative effects of aromatase inhibitors whilst not compromising statistical power. By allocating more patients to the treatment group more information on the individual biological perioperative effects of these drugs will be available, in particular with respect to assessing how these effects reflect long term outcome.

16.4 Sample Size

The number of first relapses observed in this trial determines its ability to detect differences in outcome and defines the trial size. The relapse rate is expected to be low (8-10%), hence with approximately 4350 patients we expect to observe 320-400 first relapses - during the first 5 years of follow-up. As there are two primary aims the effect on each endpoint of this sample size is detailed below:

i) Time to Recurrence

If the true five year relapse rate in patients treated with immediate surgery is 10% then with 4350 patients it will be possible to detect a 3% improvement to 7% with 91% power (two sided alpha of 5%).

In order to allow for underestimation of the relapse rate potentially due to patients dying from other causes prior to breast cancer relapse, the original sample size of 4,000 patients has been increased by a further 350 patients (taking the total sample size to approximately 4,350 patients, plus the number required to complete the POETIC sub-studies).
ii) Comparison of the prognostic value of Ki67 at surgery and baseline in the perioperative therapy group:
It will be possible to detect a 1.3 fold difference in the ability of Ki67 to predict time to recurrence, e.g. from 1.5 at baseline to 2.0 (1.5 x 1.3) or more at surgery (90% power, two sided 5% significance level). However, this will be an over-estimate of the detectable difference because 2 week and baseline Ki67 are correlated (R squared 30%), hence there would be expected to be some correlation between their ability to predict recurrence.

In addition to the primary endpoints, consideration has been given to the ability to identify molecular signatures as prognostic factors for outcome. No formal sample size considerations will be supplied to address this question. However, identifying molecular signatures requires a large amount of data. Paik et al (14), for example, used data from 447 patients to select a panel of 21 genes from a set of 250 candidate genes; this panel was then tested in a series of 668 patients. There are good grounds for recommending that the current trial employs more than 1100 patients to address this question because the molecular signature will also incorporate gene expression changes. Paik et al also found that poor grade (versus good or intermediate grade) was a significant independent prognostic factor in multivariate analysis including their recurrence score. We will be able to test the effect of both grade and Ki67 as independent factors to molecular signatures determined in this study.

16.5 Analysis Plan

Time to Recurrence (TTR) is defined as time from randomisation to local, regional, or distant tumour recurrence or death from breast cancer without prior notification of relapse. Second primary cancers and intercurrent deaths will be treated as censoring events. Patients who are alive and disease free will be censored at the date last seen alive.

Intention to treat comparisons will be tested as above with and without adjustment for centre, choice of aromatase inhibitor and known prognostic factors.

Overall survival will be measured from date of randomisation to date of death from any cause.

A sensitivity analysis endpoint will look at breast cancer free survival and relapse free survival (i.e. local, regional or distant tumour recurrence, or breast cancer death). Second primary cancers and deaths from non-breast cancer causes in the absence of breast cancer relapse will be treated as censoring events.

Analyses will be based on the intention to treat principle. For the comparison between perioperative versus no perioperative treatment the principal analysis will be a logrank comparison. Comparison of the predictive value of Ki67 at surgery in the perioperative therapy and non perioperative therapy groups will be undertaken using Cox regression, comparing the estimates of the hazard ratios obtained in each treatment group. Sample size considerations have been based on a linear relationship between the log hazard ratio and log Ki67 but as an

exploratory analysis the optimum relationship will be obtained by fitting centred linear, quadratic and cubic relationships (on three degrees of freedom). Similar methods will be used for the comparison of the predictive value of Ki67 at surgery and baseline in the AI comparison between the two timepoints will then involve comparing the change in the log-likelihood.

Cox regression methods will also be used for multivariate analyses (to further adjust for clinical factors likely to influence prognosis) and to estimate the hazard ratio and its associated confidence intervals and to test for interactions. The proportional hazards assumption will be checked to validate the use of Cox regression. The proportion of patients relapse free and surviving according to follow up time will be presented as Kaplan-Meier survival curves with fixed term survival estimates. Baseline characteristics will be described by randomised treatment group and correlations between baseline characteristics and biological markers will be investigated using Spearman Rank correlation or a chi-squared test. Comparisons will be performed using simple parametric, exact, non-parametric or chi-squared tests as appropriate. Tests will be two-sided and 95% confidence intervals will be used.

Data on compliance with treatment allocation and actual duration of hospital inpatient stay for surgery will be collected and compared between the groups.

16.6 Interim Analyses and role of Independent Data Monitoring Committee (IDMC)

An IDMC will be instigated to monitor the progress of the trial. It will meet in confidence at regular intervals, and at least annually. A report of the findings and recommendations will be produced following each meeting. This report will be submitted to the Trial Management Group and Trial Steering Committee, the main REC and the MHRA, as required.

Interim review of side effects, time to recurrence, relapse free and overall survival for all randomised patients will be performed at approximately yearly intervals. These analyses will be supplied in strict confidence by the trial statistician to the IDMC together with any other analyses that the IDMC may request. No results on survival or time to recurrence or biomarkers will be made available to investigators or any other party until at least two years after the last patient is entered or sufficient events have been observed to allow primary analysis to be undertaken, unless the IDMC determines that it would be unethical to withhold the interim results.

The main criterion for early stopping of the trial by the Trial Steering Committee, upon advice from the IDMC and endorsement from the Trial Management Group, will be that evidence from the trial and from other sources suggests a) proof beyond reasonable doubt that for all, or for some types of patient, one treatment regimen is clearly indicated or contra-indicated in terms of a net difference in relapse free and/or overall survival or toxicity, and b) evidence exists that might reasonably be expected to influence routine clinical practice. Criterion for the above will usually be a difference in time to recurrence, relapse free and overall survival at any stage significant at $p < 0.001$ (Haybittle-Peto interim stopping rule) by overall log-rank analysis. Use of this criterion will not materially affect the overall alpha in the final analysis.

The IDMC will, however, reserve the right to release any data on outcome or side-effects through the Trial Steering Committee to the Trial Management Group (and if appropriate to participants) if it determines at any stage that the combined evidence from this and other studies justifies it.

17. Study organisation

ICR-CTSU will be responsible for the collection of trial data, and for monitoring receipt and transfer of biological specimens. On receipt at ICR-CTSU, completed CRFs will be reviewed for data anomalies and their receipt recorded. Any data queries arising from initial review will be sent to the relevant centre for resolution. Following initial review, data items from the CRFs are entered centrally into the clinical study database at ICR-CTSU. Transfer of biological specimens will be monitored using a system of tracking forms sent by centres to inform ICR-CTSU of the movement of all biological samples. The Department of Academic Biochemistry at Royal Marsden Hospital will notify ICR-CTSU of all samples received. All data will be handled, computerised and stored in accordance with the Data Protection Act 1998.

17.1 Data monitoring plan

Data monitoring will primarily be conducted using central statistical monitoring, and any systematic inconsistencies identified may trigger monitoring visits to centres. Further monitoring visits may be conducted at the request of participating centres, or by random selection. Monitoring visits will be conducted at a random sub-set of participating centres and the extent and timing of this exercise will be determined by central statistical monitoring.

18. Follow-up management and completion of Case Report Forms (CRFs)

CRF completion guidelines are contained in the Trial Guidance Notes. The Trial Management Group reserves the right to amend or add to the CRFs as appropriate. Such changes do not constitute a protocol amendment, and revised or additional forms should be used by centres in accordance with the guidelines provided by ICR-CTSU.

18.1 Follow-up

Annual follow-up data will be collected for as long as the Trial Management Group consider it is contributing to the research question and will include as a minimum sites of recurrence, time of recurrence, mortality, and cause of death. Information on second primary breast cancers and other second primary tumours will also be recorded. Regular (annual or bi-annual) imaging of the breasts (e.g. mammography or MRI) should be part of the follow-up protocol according to local practice.

18.2 Relapse

The date of relapse is taken as the date of first confirmed recurrence by an appropriate investigation such as cytology, histology, or imaging wherever possible. In the absence of such confirmation, the date of first clinical suspicion will be taken provided that suspicion leads to a change or re-introduction of anti-cancer therapy. The management of recurrence will be at the discretion of the clinician. Follow-up information should continue to be provided until the patient dies.

19. Patient Protections and Ethical considerations

The study has been approved by London - South East Research Ethics Committee. Before entering patients, the Principal Investigator at each participating centre is responsible for gaining Site Specific Assessment and Research and Development approval.

Patients entering the study via Pathway A will be asked to sign *either* the generic consent form approved by London - South East Research Ethics Committee for use within POETIC, *or* a consent form approved by a Research Ethics Committee for the tissue bank holding tissue pending entry to POETIC, provided a copy of the information and blank consent form have been approved by the Chief Investigator. Prior to entering POETIC patients should be asked to sign the POETIC consent form after receiving both verbal and written information. This form incorporates consent to the biological studies. All consent forms must be countersigned by the Principal Investigator or a designated individual. A record of who the designated individuals are and the circumstances under which they may countersign consent forms must be clearly documented at the research site as part of the Delegation Responsibilities Log. This log, together with original copies of all signed patient consent forms, must be available for inspection.

The POETIC patient information sheet should be provided in addition to any standard patient information sheets for aromatase inhibitors that are provided by the centre and which are used in routine practice.

19.1 Liability/Indemnity/Insurance

Indemnity for participating hospitals is provided by the usual NHS indemnity arrangements.

19.2 Patient Confidentiality

The patient's full name, date of birth, hospital number and NHS number (CHI number in Scotland) will be collected at randomisation to allow tracing through GP and national records to assist with the collection of long term follow-up information. The personal data recorded on all documents will be regarded as confidential, and any information which would allow individual patients to be identified will not be released into the public domain.

The investigator must keep a separate log of patients' trial numbers, names, addresses and hospital numbers. The investigator must maintain trial documents, which are to be held at the participating centre (e.g. patients' written consent forms), in strict confidence. The investigator must ensure the patients' confidentiality is maintained.

ICR-CTSU will maintain the confidentiality of all patient data and will not reproduce or disclose any information by which patients could be identified. Representatives of ICR-CTSU and the regulatory authorities are required to have access to patient notes for quality assurance purposes. Patient confidentiality will be respected at all times. (In the case of special problems and/or government queries, it is also necessary to have access to the complete study records, provided that patient confidentiality is protected).

20. Withdrawal of patients from the trial treatment

20.1 Withdrawal of patients from trial treatment

Patients who do not receive their allocated treatment for any reason should be treated at the discretion of their clinician. Unless the patient requests otherwise, all CRFs, including long term follow-up, should be completed, regardless of treatment actually received, as analyses of all outcome efficacy data will be on the basis of intention to treat (i.e. all randomised patients). A trial deviation form should be completed to record details of deviation from treatment allocation. Patients are asked prior to randomisation to consent to follow-up should they withdraw from their allocated treatment (see patient information sheet and consent form), and any patient unwilling to give that assurance prior to trial entry should not be randomised. Patients are, however, free to reverse that decision at any time without giving a reason (see below).

20.2 Withdrawal of patients from trial follow-up

A trial deviation form should be completed in the unlikely event that the patient withdraws consent for further follow-up data to be collected. If this situation is suspected, clarification should be sought to ensure that the patient is not simply withdrawing from allocated treatment (as above). In the extremely unlikely event that the patient wishes to have their data removed from the trial completely (the implications of this should be discussed with the patient to ensure that this is their intent) this should be indicated as such on the trial deviation form.

21. Completion of the study and definition of study end date

The study end date is deemed to be the date of last data capture.

22. Research Governance

22.1 Trial Administration

The Co-sponsors of the POETIC trial are The Institute of Cancer Research (ICR) and the Royal Marsden NHS Foundation Trust (RMH), the Chief Investigator's host institution. Sponsorship activities and delegated responsibilities are shared between RMH and ICR, in accordance with The Medicines for Human Use (Clinical Trials) Regulations 2004 as amended and in line with the Research Governance Framework for Health and Social Care and the principles of GCP. Both parties agree to allow inspection of their premises by the competent authorities. Responsibilities of the Co-sponsors are set out in an agreement letter between ICR and RMH.

22.1.1 Trial Administration - Royal Marsden Hospital Responsibilities

RMH has sponsorship responsibility for obtaining authorisation and appropriate ethics committee opinion (Part 3 of the Regulations) and for pharmacovigilance (Part 5 of the Regulations).

The following responsibilities have been delegated:

The Chief Investigator:

- Selection of investigators

To the Chief Investigator or a named deputy delegated in his absence:

- Prompt decision as to which serious adverse reactions are SUSARs; and
- Prompt reporting of that decision to ICR-CTSU, Section of Clinical Trials, The Institute of Cancer Research for onward reporting to the licensing authority.

To The Institute of Cancer Research (through ICR-CTSU):

- Ensuring an appropriate ethics opinion has been sought, and any amendments have been approved;
- Giving notice of amendments to protocol, make representations about amendments to the main REC;
- Giving notice that the trial has ended;
- Keeping records of all serious adverse events (SAEs) reported by investigators;
- Ensuring recording and prompt reporting of serious adverse reactions (SARs) to the Chief Investigator;
- Reporting to the MHRA and main REC any suspected serious adverse reactions which the Chief Investigator considers to be unexpected (SUSARs);
- Ensuring Principal Investigators are informed of all SUSARs;
- Providing all SUSARs, including those in third countries, to the MHRA for inclusion on the European database; and
- Providing an annual list of SARs and a safety report to the licensing authority and Sponsors.

To participating centres:

- Ensuring recording and prompt reporting of SAEs/SARs to ICR-CTSU, Division of Clinical Studies, The Institute of Cancer Research.

22.1.2 Trial Administration - The Institute of Cancer Research Responsibilities

ICR has responsibility for ensuring the trial is conducted in accordance with the principals of Good Clinical Practice (Part 4 of the Regulations).

The following responsibilities have been delegated:

To RMH:

- Taking appropriate urgent safety measures – delegated to the Chief Investigator.

To participating centres:

- Putting and keeping in place arrangements to adhere the principles of GCP;
- Keeping a copy of all ‘essential documents’ (as defined under the principles of GCP) and ensuring appropriate archiving and destruction of documentation once the trial has ended as required by regulation 31 of the principal Regulations of the Medicines for

Human Use (Clinical Trials) Regulations 2004 implementing the commission directive 2005/28EC;
- Ensuring investigational medicinal products (IMPs) are made available to subjects free of charge; and
- Taking appropriate urgent safety measures.

Responsibilities are defined in an agreement between an individual participating centre and the co-sponsors.

ICR is responsible for administering funding and co-ordinating any required legal agreements and investigator statements.

The delegation of sponsorship responsibilities does not impact on or alter standard NHS indemnity cover. The agreement of delegated responsibilities is viewed as a partnership and as such it is necessary to share pertinent information between ICR and RMH/Chief Investigator, including proposed inspections by the MHRA and/or other regulatory bodies.

22.2 Protocol Compliance & Initiation

The POETIC trial is being conducted in accordance with the professional and regulatory standards required for non-commercial research in the NHS under the EU Directive. Before activating the trial, participating centres are required to sign an agreement accepting responsibility for all trial activity which takes place within their centre.

Sites may commence recruitment once centre agreements have been signed by both parties, trial documentation is in place, and a telephone site initiation has taken place. Site initiation visits will be conducted at sites where the Principal Investigator has requested one, or where ICR-CTSU thinks that it is appropriate. Site initiation visits are not considered necessary for sites that have experience with other ICR-CTSU managed phase III breast cancer trials, or where staff have attended the Investigator Launch meeting.

22.3 Data Acquisition & On-Site Monitoring

On-site monitoring, or auditing, will be based on a risk-based strategy. ICR-CTSU staff may visit centres to confirm that agreements are being adhered to, specifically to carry out source data verification and confirm compliance with the protocol and the protection of patients' rights as detailed in the Declaration of Helsinki 1964 as amended October 1996. By participating in the POETIC trial, the Principal Investigators at each centre are confirming agreement with his/her local NHS Trust to ensure that:

- sufficient data is recorded for all participating patients to enable accurate linkage between hospital records and CRFs;
- source data and all trial related documentation are accurate, complete, maintained and accessible for monitoring and audit visits;
- all staff at their centre who are involved with the trial will meet the requirements of the EU Directive;

- original consent forms are dated and signed by both patient and investigator and are kept together in a central log together with a copy of the specific patient information sheet(s) given at the time of consent;
- copies of CRFs are retained for 15 years to comply with international regulations; and
- staff will comply with the protocol and Trial Guidance Notes for the POETIC trial.

ICR-CTSU will monitor receipt of CRFs and evaluate incoming CRFs for compliance with the protocol, inconsistent or missing data.

Participating centres may be monitored by ICR-CTSU and possibly by Health Authorities. Monitoring by ICR-CTSU aims to verify compliance with the protocol and to conduct source data verification (SDV).

Site monitoring will be conducted at a proportion of participating centres at least once during the course of the trial. If a monitoring visit is required ICR-CTSU will contact the centre to discuss dates of proposed visit. Once a date has been confirmed a list of patients whose notes will be monitored during the visit will be sent to the centre. This list will be sent out in advance to give sufficient time for the notes to be made available. The Trial Statistician will decide what percentage of patients is to be monitored.

If any problems are detected in the course of the monitoring visits then the Principal Investigator and ICR-CTSU will work together to resolve issues and, if necessary, to determine the centre's future participation in the study.

22.4 Archiving

Essential trial documents should be retained according to local policy and for a sufficient period for possible inspection by the regulatory authorities (at least 5 years after the date of last data capture). Documents should be securely stored and access restricted to authorised personnel.

22.5 Data Protection Act (DPA)

ICR-CTSU will comply with all aspects of the DPA 1998. Any requests from patients for access to data about them held at ICR-CTSU should be directed to the Trial Manager in the first instance, who will refer the request to the Data Protection Officer at The Institute of Cancer Research.

22.6 Financial Matters

The trial is investigator designed and led, and has been approved by Clinical Trials Advisory & Awards Committee (CTAAC) of Cancer Research UK, and meets the criteria for R&D support as outlined in the Statement of Partnership on Non-Commercial R&D in the NHS in England.

The trial has received funding from Cancer Research UK. If additional financial support is received from any other source this will be made apparent to the approving Main REC and CTAAC, but will not require a protocol amendment.

No individual per patient payment will be made to Trusts or investigators, but NCRN (or regional equivalent) network resources should be made available, as the trial is part of the NIHR portfolio.

22.7 Clinical risk assessment

Generic Risk Assessment Hazards to patients, study and organisation have been performed for the POETIC trial.

23. Publication policy

The main trial results will be published in the name of the trial in a peer-reviewed journal, on behalf of all collaborators. The manuscript will be prepared by a writing group, appointed from amongst the Trial Management Group, and high accruing clinicians. All participating centres and clinicians will be acknowledged in this publication together with staff from ICR-CTSU. All presentations and publications relating to the trial must be reviewed and approved by the Trial Management Group on whose behalf publications should usually be made. Authorship of any secondary publications, e.g. relating to the various biological studies, will reflect the intellectual and time input into these studies, and will not be the same as on the primary publication. No investigator may present or attempt to publish data relating to the POETIC trial without prior permission from the Trial Management Group.

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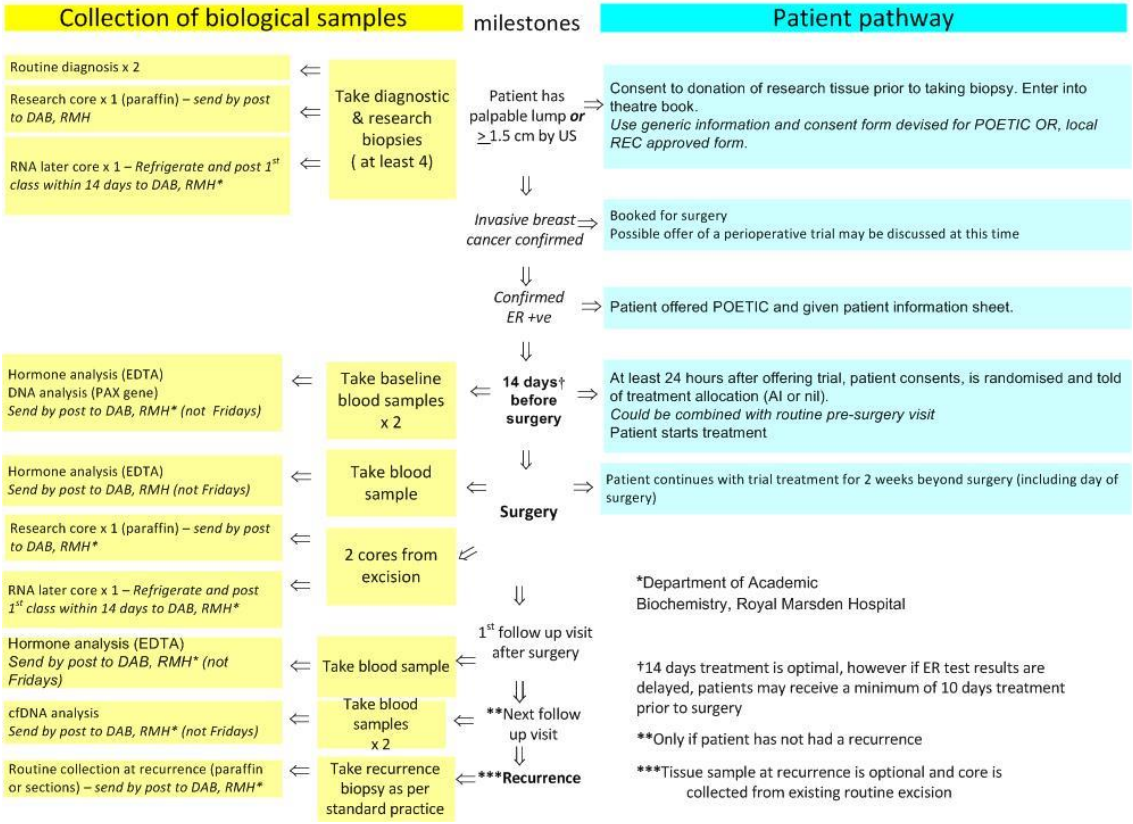
Appendix 1 Glossary of Terms

AI	Aromatase inhibitor
BASO	British Association of Surgical Oncology
ctDNA	circulating tumour DNA
CI	Chief investigator
CRF	Case Report Form
CTA	Clinical Trials Authorisation
CTAAC	Clinical Trials Advisory and Awards Committee
CXR	Chest X-Ray
DNA	DeoxyriboNucleic Acid
DPA	Data Protection Act
EBCTCG	Early Breast Cancer Clinical Trialists' Collaborative Group
ER	Oestrogen receptor
EU	European Union
FBC	Full Blood Count
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GP	General Practitioner
HER2	Human Epidermal growth factor Receptor 2
HPV	Healthy postmenopausal volunteer
HRT	Hormone Replacement Therapy
HTA	Human Tissue Authority
ICR-CTSU	The Institute of Cancer Research Clinical Trials and Statistics Unit
IDMC	Independent Data Monitoring Committee
IHC	Immunohistochemistry
IMPs	Investigational Medicinal Products
ISRCTN	International Standard Randomised Controlled Trial Number
Main REC	Main Research Ethics Committee
MHRA	Medicines & Healthcare products Regulatory Agency
NCRI	National Cancer Research Institute
NCRN	National Cancer Research Network
NIHR	National Institute for Health Research
OS	Overall Survival
PK	Pharmacokinetics
PgR	Progesterone receptor
RFS	Relapse Free Survival
RMH	Royal Marsden NHS Foundation Trust
RNA	RiboNucleic Acids
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SNB	Sentinel Node Biopsy
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee

WHO	World Health Organisation

Appendix 2 - Patient Pathways

POETIC Study
Patient Pathway A, and timelines for tissue sample collection
Identify postmenopausal patients with high likelihood of cancer



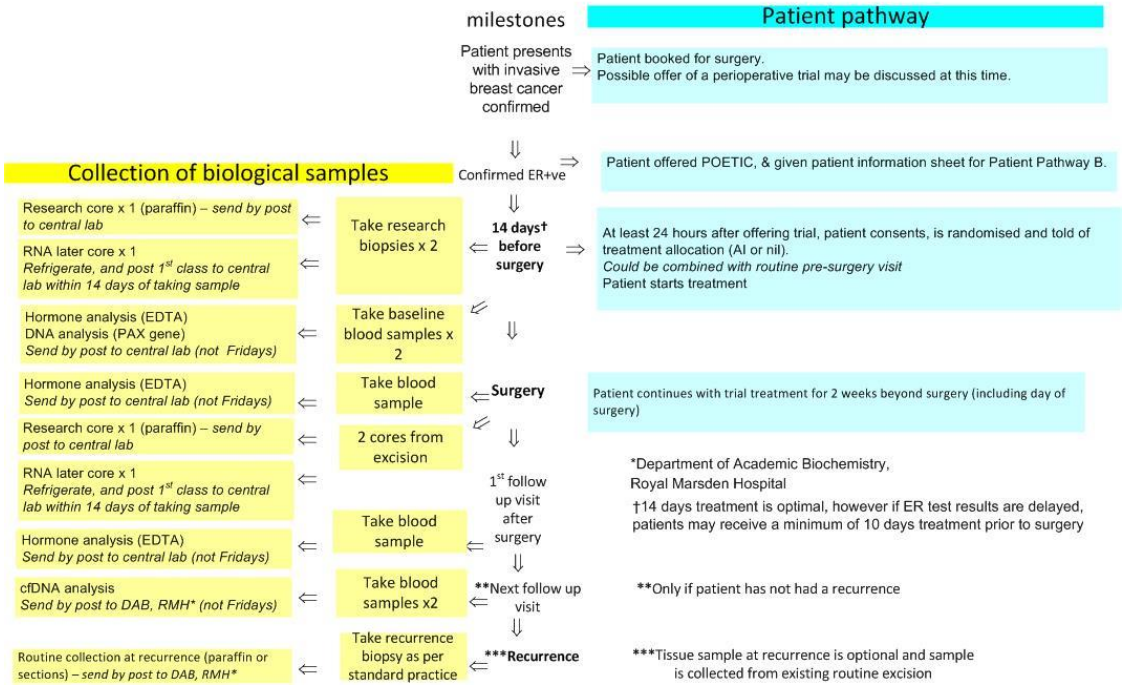
*Department of Academic Biochemistry, Royal Marsden Hospital

†14 days treatment is optimal, however if ER test results are delayed, patients may receive a minimum of 10 days treatment prior to surgery

**Only if patient has not had a recurrence

***Tissue sample at recurrence is optional and core is collected from existing routine excision

POETIC Study
Patient Pathway B, and timelines for tissue sample collection
Identify postmenopausal patients with invasive cancer confirmed



Appendix 3 – Tissue sample collection and analysis

A. Sample collection:

Tissue samples for patients entering POETIC from Biological Centres:

At diagnosis (pathway A) or immediately before randomisation (pathway B): 2 core biopsies (14-guage) are required ***in addition*** to those taken for diagnostic purposes. One is to be placed into formalin and the other placed in RNA-later® in tubes provided. All formalin fixed biopsies will be embedded in paraffin wax at the local histopathology department. If possible samples in RNA-later® should be refrigerated overnight at 4°C to allow thorough penetration of the tissue. If refrigeration is not available, samples can be stored at 25°C in RNAlater® Solution for up to 1 week without significant loss of RNA quality.

a. The sample in RNA-later® and the paraffin block, without prior sectioning, are to be sent by post to the Department of Academic Biochemistry, Royal Marsden Hospital either immediately the patient enters POETIC or together with samples taken at surgery (see below).
b. **At surgery:** 2 core biopsies (14-guage) to be taken immediately the tumour has been excised. One is to be placed into formalin and the other placed in RNA-later® in tubes provided. All formalin fixed biopsies will be embedded in paraffin wax at the local histopathology department. Samples in RNA-later® are to be stored as above, and both samples sent by post to the Department of Academic Biochemistry, Royal Marsden Hospital as soon as possible.

Kits for the collection of samples in RNA-later® are provided. Packaging, labelling and tracking is to be carried out in accordance with the Trial Guidance Notes.

Tissue samples from patients entering POETIC from all other centres

Tissue must be provided for all patients using one of the three options below. The option chosen should be on a case by case basis; however the type of tissue provided from diagnosis should be the same as that provided at surgery. The options for provision of tissue are:

a. **One tissue block of the diagnostic and excision samples**. This is the preferred option. From each of these, 10 thin (3 micron) section and 4 thick (10 micron) sections will be taken. Sections will be taken such as not to exhaust the blocks, which will be returned to the original pathology laboratory.
b. **Provision of the above sections on slides**, cut by the originating pathology laboratory
c. **2 thin (3 micron) sections.** This is the minimum for the patient to be eligible.

Tissue sample from all patients all pathways:

At the time of recurrence, an additional FFPE tissue block or sections, from routine excision at the time of recurrence, are optionally collected.

Blood samples – all centres
All centres must participate in the collection of blood samples (although individual patients may still enter POETIC and decline donation of blood samples):

a. **At randomisation:** 2 blood samples; one in EDTA and one in PAXgene (tubes provided).
b. **Up to 24 hours before surgery:** one blood sample in EDTA.
c. **At first follow-up visit after surgery:** one blood sample in EDTA.
d **Late follow-up visit:** 2 blood samples; one in EDTA and one in Streck (not required if patient has relapsed).

All blood samples are to be posted to the Department of Academic Biochemistry, Royal Marsden Hospital on the same day the sample is taken using the blood kits provided. Sample collection, packaging, labelling and tracking are to be carried out in accordance with the Trial Guidance Notes.

Patient consent procedures provided in the Trial Guidance Notes should be followed.

B. Sample analysis:

The formalin fixed core biopsies will be sectioned for the analysis of the proliferation marker, Ki67, by immunohistochemistry. Other sections will be stored after coating with paraffin wax for assessment of additional biomarkers that are candidates as being involved in response or resistance to hormonal treatment or in other aspects of breast cancer biology.

The core biopsies contained in RNA-later® will be analysed for RNA profiles and/or changes in the DNA of the tumour.

The EDTA-preserved blood samples will be centrifuged and the plasma stored frozen for the analysis of oestrogen levels. Residual plasma will be stored frozen for the analysis of biomarkers that may be related to the efficacy of treatment or the prognosis of the patient.

The blood samples collected into Streck tubes will be centrifuged and the supernatant stored frozen for the estimation of circulating tumour DNA.

The PAXgene-preserved blood sample will be stored for future analysis of germ-line DNA in relation to disease outcome, biological response (e.g. change in Ki67) to the aromatase inhibitor or tolerability of treatment.

Appendix 4 – WHO Performance Status

0	Normal activity
1	Strenuous activity restricted, can do light work
2	Up and about ≥ 50% waking hours, capable of self care
3	Confined to bed > 50 waking hours, limited self care
4	Confined to bed or chair, no self care, completely disabled

Appendix 5 - Patient information sheet and consent forms

For patient pathway A: patients should be offered either the POETIC generic information and consent form approved by Main REC, or generic patient information and consent forms approved by an ethics committee specifically for patients donating tissue to that tissue bank. Locally approved information and consent must be adequate to allow tissue to be used within POETIC. All patients who have donated tissue at diagnosis and invited to take part in POETIC must be offered the POETIC information sheet for Pathway A

For patient pathway B: patients must be offered the POETIC information sheet for Pathway B.

For patient at non-biological centre: patients must be offered the POETIC information sheet for non-biological centres.

Additional information and consent form for patients taking part in the POETIC study: additional information and consent form for patients taking part in the POETIC study version 1 - 09 May 2011 approved by Main REC. Patients consented to the POETIC patient information sheet and consent form Versions 1-3 (Version 1 – 12 March 2008, Version 2 – 01 August 2008, or Version 3 – 22 December 2009) approved by Main REC are/were asked to re-consent at their next visit to give permission for the extraction, storage and research on genetic material (DNA) in blood samples already donated.

Additional consent for late follow up blood samples and tissue at recurrence sample collection: patients should be invited to consent to the additional samples collection (late follow up blood and tissue at recurrence sample) ideally at their next routine or at an additional visit. Centres should contact participants in line with their local practice. Should the centres contact the patients initially via post, the letter provided to accompany the consent for additional samples collection may be used,

All patient information sheets and consent forms are provided as separate documents. Further guidance on gaining patient consent is provided in the Trial Guidance Notes.